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A CORRELATIVE STUDY OF THE CLINICAL, PATHOLOGICAL AND MOLECULAR BIOLOGICAL FEATURES OF CREUTZFELDT-JAKOB DISEASE

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To IB, RGW and CPW, my mentors in Neurology

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This work would not have been possible without the guidance and assistance of a large number of individuals, only some of whom are mentioned here by name. This is particularly true in relation to the relatives of the patients studied here. Their eagerness to assist me, in frequently tragic circumstances, was astonishing, not to mention their acts of kindness and hospitality. I hope that this research is of some small consolation. Also not mentioned individually are colleagues in Neurology, Neurophysiology and Neuropathology all over the UK, who referred cases and gave unstintingly of their time when I was in their units collecting data.

The following were my partners in crime: Bob Will, Tom Esmonde, Martin Zeidler, James Ironside, Jeanne Bell, Ken Sutherland, Ian Goodbrand, Jan MacKenzie, Sandie Honeyman, Linda McCardle, Caroline Barrie, Dave Nicholson, Otto Windl, Maureen Dempster, Peter Estibeiro and Jim Slattery.

DECLARATION

I, Rajith N. de Silva, hereby confirm that this thesis has been composed by myself. The work undertaken was part of a research effort by several individuals, but my contribution to the data collection and analysis presented here was substantial; the contribution of others to this work has been clearly indicated.

LIST OF ABBREVIATIONS

CJD	Creutzfeldt-Jakob disease
PRNP	Human prion protein gene
ORF	Open reading frame (of gene)
ATD	Dementia of Alzheimer type
PrP	Prion protein
BSE	Bovine spongiform encephalopathy
HGH	Human growth hormone
HGnH	Human gonadotrophin
FFI	Fatal familial insomnia
EEG	Electroencephalogram
GSS	Gerstmann-Sträussler syndrome
NIH	National Institutes of Health
CT	Computerised tomography
CSF	Cerebrospinal fluid
MRI	Magnetic resonance imaging
LMN	Lower motor neurone
PrP^{c}	Protease-sensitive PrP
PrP^{Sc}	Protease-resistant, "infective" PrP
Met	Methionine
Val	Valine
APOE	Apolipoprotein E
vCJD	New variant CJD
PCR	Polymerase chain reaction
SSCP	Single-strand conformational polymorphism
OPCS	Office of population, census and statistics

A B S T R A C T

A systematic study of all patients with pathologically or electrophysiologically confirmed Creutzfeldt-Jakob disease (CJD) referred to the U.K. national surveillance unit between May, 1990 and April, 1994 has been undertaken. The numbers of sporadic, familial and iatrogenic cases were 144, 14 and 12 respectively. Sporadic CJD cases had a median age at presentation of 65 years, and a median disease duration of 4 months. Familial cases (associated with mutations of the open reading frame of the prion protein gene, PRNP ORF) presented 10 years earlier and had disease durations which were twice as long. Clinical characteristics at different stages of illness were identified. At presentation, around 40% of sporadic cases had some aspect of cognitive impairment in isolation, 30% had cerebellar dysfunction in isolation, 10% had a combination of cognitive and cerebellar dysfunction, and 10% had occipital blindness. Alternative modes of presentation were unusual (<10%). In most of the 30% of sporadic cases that had cerebellar dysfunction as their presenting feature, other neurological abnormalities quickly supervened; the form of CJD characterised by *pure* progressive cerebellar ataxia was extremely rare (<4%). The clinical characteristics of the sporadic and the grouped familial cases did not differ. Characteristic electroencephalographic findings were present in 35% of pathologically confirmed cases. Familial cases were more likely to have a family history of (non-specific) neurodegeneration. Iatrogenic cases in whom the agent was inoculated outside the central nervous system were shown to have a different clinical profile early in the illness from sporadic cases.

The clinical characteristics of the sporadic and familial cases were compared at different stages of illness with those of a group of patients with suspected CJD

whose neuropathological examinations had revealed an alternative neurodegenerative process (non-CJD). The relative sensitivities and specificities of the standardised criteria used in the clinical evaluation of suspect CJD cases were high, with the exception of "neurogenic muscle wasting". The non-CJD group had a disease duration that was four times as long as the sporadic CJD cases. In this group, Alzheimer-type dementia was the commonest diagnosis with a large proportion having myoclonus.

The correlation of clinical features with spongiform change distribution in different parts of the brain was in general poor. Various explanations for this discrepancy have been offered. Around 15% of sporadic CJD cases were found to have unicentric anti-PrP positive amyloid plaques. These cases were more likely to be associated with alleles encoding valine at position 129 of the PRNP ORF.

The vast majority of sporadic CJD cases were methionine homozygous at codon 129 of the PRNP ORF. The ages of onset and disease durations between patients who were homozygous and heterozygous at this site of common polymorphism did not vary. Patients who bore alleles encoding valine at this site (as well as demonstrating amyloid plaques) were more likely to present with cerebellar ataxia and were unlikely to have typical electroencephalographs. Despite the incurable and devastating nature of CJD, relatives were keen to know the results of PRNP genome analysis in patients.

A comprehensive classification of human spongiform encephalopathy as understood in the early 1990s has been presented. This constitutes a

background against which probable novel forms of human spongiform encephalopathy (such as new variant CJD) can be contrasted.

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DISCUSSION

INTRODUCTION

Creutzfeldt-Jakob disease (CJD) is a rare and invariably fatal neurodegenerative disease, characterised by spongiform change of the central nervous system and transmissibility. It is one of a group of disorders affecting a variety of mammalian species (Table 1). In humans, the illnesses traditionally associated with spongiform encephalopathy are sporadic CJD (with no known causation), familial CJD (attributable to inherited factors), iatrogenic CJD (due to medical "misadventure") and kuru. Despite their rarity (the annual incidence of sporadic CJD worldwide is 0.5 to 1.0 cases/million population/year),[1] these disorders have attracted an extraordinary amount of lay interest. The epidemic of Bovine Spongiform Encephalopthy (BSE)[2] has fuelled speculation on the impact of this animal spongiform encephalopathy on humans. Amongst scientists, interest in the spongiform encephalopathies arises due to two reasons. Firstly, these disorders straddle the entire spectrum of mechanisms by which human disease is traditionally thought to be caused. On the one hand CJD can arise from purely genetic factors and on the other the illness is apparently purely environmentally acquired (for example, in recipients of "infected" pituitary-derived human growth hormone [HGH][3]). Secondly, the nature of the putative "agent" of infectivity has remained remarkably difficult to characterise, being apparently devoid of nuclear material and being denatured by proteolytic agents only.[4]

The prion hypothesis has emerged as a credible explanation for some of these unusual features. In this, it is argued that the vector of infectivity is a conformational isoform of a naturally occurring protein (prion protein [PrP]), with a capacity to convert normal PrP to its abnormal isoform autocatalytically. In humans, the gene encoding PrP (*PRNP*) is located on the short arm of chromosome 20. Familial forms of CJD have been associated with mutations in *PRNP*, and a site of common polymorphism appears to render increased susceptibility to iatrogenic, sporadic and possibly new

variant CJD (vide infra). The normal rôle of PrP has not been fully elucidated. In early PrP-null transgenic mouse models, no abnormalities were noted,[145] but later experiments have revealed abnormal sleep patterns,[147] altered electrophysiological characteristics[146] and possibly accelerated cerebellar Purkinje cell loss.[211]

The clinical manifestations of CJD are protean, and there are no consistently reliable diagnostic tests. These factors contribute to its diagnostic difficulty. Within the diverse clinical signs, however, patterns are recognised, and forms of the illness characterised by occipital blindness[5] and progressive cerebellar ataxia[6] are recognised. The improved diagnosis of familial forms of CJD, frequently utilising *PRNP* genome analysis, has broadened the spectrum of clinical features associated with CJD to include atypical characteristics, such as dysautonomia.[7] Furthermore, in iatrogenic CJD and especially where the agent of infectivity is introduced outside the central nervous system, the clinical phenotype is remarkably homogeneous.[8]

CJD is associated with distinct neuropathological features. In addition to microscopic spongiform change, there is astrocytosis and neuronal cell loss.[9] In a proportion of cases, there are abnormal amyloid protein aggregates-termed plaques. Immunocytochemistry with anti-PrP antibodies has revealed positive staining of these lesions, and also within and around areas of confluent spongiosis.[10]

The current study is an exploration of the clinical phenotypes associated with CJD, based on cases referred to the UK national CJD surveillance unit up to 1994. The unit was set up in 1990, following the recommendation of the Southwood committee, which reported to Parliament on the likely impact of BSE.[12] In addition to collecting epidemiological data on a variety of putative

"risk factors", detailed clinical profiles of patients referred to the unit are available. As the route of inoculation in iatrogenic CJD may be crucial in determining the clinical presentation, human exposure and susceptibility to BSE might result in an altered clinical phenotype. The detailed clinical profiles are used to describe the frequency of common and unusual clinical signs in cases during the period of study. These profiles are compared with those of a group of "control" patients, also referred to the unit with suspected CJD, but whose neuropathological examination did not confirm CJD. In those cases with neuropathological data, the clinical phenotypes are correlated with pathological appearances (in particular spongiform change). Clinico-pathological correlation is also attempted in cases with amyloid plaques. Data on *PRNP* genotype is available in a proportion of cases, and in these correlation with clinical (and, where available, pathological) features is performed. Ethical considerations in relation to *PRNP* genome analysis are also explored.

1.1 Early clinical descriptions

H.G. Creutzfeldt (Figure 1) is credited with the first description of the disorder, although by current diagnostic criteria his case is atypical.[13] The patient was twenty-three years at presentation and there was a questionable family history of mental subnormality. In adolescence the patient was noted to be immature and had behavioural abnormalities. A year prior to presentation, she was treated for a skin rash and at that time spastic paraparesis was recorded. The latter improved thereafter, but there were features of psychiatric disturbance, cerebellar ataxia and possibly dystonia at the time of presentation. During her terminal hospitalisation, her behaviour continued to be disturbed, she exhibited a variety of cognitive deficits, pyramidal and

cerebellar signs, and probably had myoclonus. She died in status epilepticus, two months after presentation. The neuropathological findings were those of "...a noninflammatory focal disintegration at the neural tissue of the cerebral cortex with neuronophagia and reparative glial proliferation..."[13] A year later, in 1921, A. Jakob (Figure 2) described four cases, at least one of whom had clinical features suggestive of the entity we recognise as CJD now (Table 2).[14] A further case which Jakob considered similar was described in 1923.[15] Masters and Gajdusek have reviewed the pathology of Jakob's five cases, and conclude that only cases 3 and 5 have got spongiform encephalopathy.[16] They suggest that case 1 had motor neurone disease, and that cases 2 and 4 had toxic or metabolic encephalopathies. Kirschbaum had come to a similar conclusion (clinically) regarding case 4.[17]

Over the next forty years there was considerable confusion over nomenclature, with at least twelve synonyms being applied to this disorder.[18] Of these, three descriptions are worthy of further mention.

1.1.1 "Subacute vascular encephalopathy"

In 1954 and 1960, Nevin and colleagues in two elegant series described a total of ten patients, all of whom succumbed to a neurodegenerative process of subacute onset and rapid progression.[19,20] They drew attention to the combination of pyramidal and cerebellar disturbance, the presence of involuntary movements especially myoclonus, the frequent occurence of visual failure, the spectrum of speech disturbances (dysphasia, dyspraxia and mutism), the recurrence of primitive reflexes, and the rigidity ("Passive movements were resisted..."). The association of the disorder with characteristic electroencephalogram (EEG) appearances was indicated, although it was also stressed that the EEG abnormalities were not *specific.*[20] Finally, the authors gave detailed descriptions of the neuropathological

appearances, and on account of the spongiform change concluded that the aetiology of the disorder was microvascular dysfunction. Despite the erroneous attribution of causation, these descriptions remain the most comprehensive accounts of the clinical course of CJD.

1.1.2 Heidenhain's syndrome

In 1929 Heidenhain reported three cases of rapidly progressive dementia, two of whom had had blindness during the terminal phase.[21] The neuropathological features were those of CJD, but in the patients with blindness the degree of spongiform change was most pronounced in the occipital cortices (including the calcarine regions). In 1954 Meyer and colleagues reviewed Heidenhain's cases and reported a further case.[5] Meyer's case was a man aged 38 who died six months after the onset of progressive dementia accompanied by visual failure. On examination, he was severely demented, appeared to have a right homonymous hemianopia, had exaggerated limb reflexes on the left and was ataxic. On histological examination after necropsy, diffuse spongiform change affecting all cortical regions was confirmed, but once again the changes were most pronounced in the occipital lobes. In the calcarine regions the appearances were those of "status spongiosus" (similar to Heidenhain's second case). In conclusion, Meyer *et al* felt that these cases represented a variant form of CJD characterised by rapidly progressive dementia and cortical blindness, with less involvement of the pyramidal and extra-pyramidal systems. It is of note that all three patients exhibited cerebellar signs in addition.

1.1.3 Ataxic CJD

In 1965 Brownell and Oppenheimer described four patients with pathologically confirmed CJD.[6] All four had presented with cerebellar ataxia, as had six cases they reviewed from the literature. The pathological

appearances were those of CJD, with neuronal cell loss and astrocytosis being particularly noted in the cerebellum. The authors themselves drew attention to their fourth case, which had a slightly unusual course. A 60 year-old woman was seen with a six month history of difficulty walking. The key examination findings were gross limb tremor and gait ataxia. At initial assessment, she was "vague and forgetful" but fully orientated. Within the space of a month she had become disorientated and demented. By then she was unable to stand or walk without support. Terminally, she had feeding difficulties and was doubly incontinent. At the time of death, the suspected diagnosis was (idiopathic) cerebellar degeneration (lumbar puncture and air encephalography had been normal). The total duration of illness had been 8 months.

As indicated in their discussion, this case (of CJD) was unusual in having a course that was for the most part dominated by cerebellar ataxia, with dementia being a late feature. The pathological changes in the cortex of this case were slight, but the authors felt that there was no reason why "...in this variable condition, varieties should not occur in which the cerebral cortex remains more or less intact."

1.2 Gerstmann-Sträussler syndrome (GSS)

In 1928 Gerstmann described an unusual inherited cerebellar disorder. A 25 year-old woman developed ataxia, dysarthria and personality change, and on review a year later had more pronounced ataxia and dementia.[22] Over the next five years (leading to death) the patient exhibited "pseudobulbar disturbance of swallowing", nystagmus (lateral and upgaze), limitation of upgaze, hypotonia, intention tremor, diminished reflexes and bilaterally

upgoing plantar responses. In a subsequent publication,[23] the detailed clinical and pathological features of this case along with the family history (there were seven other affected members) were described. The pathological appearances were those of neuronal loss in most areas of the cerebellum and in the dentate nuclei, corticospinal and cerebellar peduncle fibre loss, cerebral cortical gliosis, and *argyrophilic plaques throughout the cerebellum, cerebrum and brainstem.* Spongiform change was present but not striking. In their seminal publication in 1981, Masters *et al.* reviewed Gerstmann's case along with nine other cases in the literature sharing similar clinical and pathological features.[24] Seven further cases with identical phenotypes referred to the authors' laboratory for transmission experiments were included in the paper. In three of the latter group, a spongiform encephalopathy had occured after inoculation of brain tissue into nonhuman primates. In the group as a whole, the mean age at death was 48 years (range 29 to 62) and the mean duration of disease was 59 months (range 13 to 132). The key clinical features were characterised as cerebellar incoordination, pyramidal signs and dementia. Myoclonus was not a constant finding. The slow evolution of the illness was felt to be the main differentiating feature of this group of patients, from patients with CJD.

In 1990 "linkage" of a missense variant at codon 102 of *PRNP* was demonstrated in GSS,[25] and in 1991 the same mutation was identified in Gerstmann's original family.[26]

1.3 Kuru

The epidemic of kuru which predominantly affected the Fore-speaking people of the highlands of Papua New Guinea, was recognised as a progressive and

eventually fatal neurodegenerative process predominantly affecting the cerebellum.[27,28] In 1979 R.W. Hornabrook presented a comprehensive review of the clinical features of this illness, based on his experience with 434 cases.[29] The most salient observation was the remarkable uniformity of clinical features in affected patients. ("A resemblance which could not be closer were they coined from the same mint."[29]) During a prodrome of twelve months or more, affected patients would show transient unsteadiness. At this time minor changes in personality and mood (mild euphoria, tendency towards fatuousness and lack of insight) may have been present. The clinical illness itself was characterised by progressively worsening ataxia and the inability to maintain balance. In the last stages patients were unable to sit or perform any activity, were grossly dysarthric and were unable to swallow. Death would ensue 12 - 18 months after the onset of the clinical illness. Dementia was probably present at the terminal stage of disease. Signs of extra-pyramidal disease, rigidity, myoclonus and seizures were absent. In children, the clinical illness was more variable and of shorter duration. Brainstem and bulbar dysfunction appeared to occur more commonly than in adults.

W. Hadlow's prescient comments on the histopathological similarities between brains affected by scrapie and kuru,[30] led to the successful transmission of kuru by intracerebral inoculation of kuru brain or mixed visceral tissues into chimpanzees and monkeys.[31] This supported the theory that endocannibalistic practices (not just consumption, but also contamination and subsequent inoculation via mucous membranes, skin and eyes) were responsible for this illness. Furthermore, there has been a dramatic fall in the number of kuru cases born after the cessation of these practices in around 1956.[32]

In the foregoing, early descriptions of CJD have been reviewed. Variant sporadic forms such as those presenting with cortical blindness and illnesses dominated by cerebellar incoordination were recognised early in the history of this disorder. Transmission of CJD to non-human primates by intracerebral inoculation was achieved in 1968, establishing the commonest human spongiform encephalopathy as a transmissible disorder (albeit under experimental conditions).[33] Rare types of human spongiform encephalopthy related to familial factors and endocannibalism have also been reviewed. Over the 1970s several large epidemiological studies were performed to investigate the possibility that sporadic CJD arose from case-to-case transmission. No evidence for this hypothesis emerged,[34-37] but in the course of these investigations a large amount of data on the clinical features of CJD was collected.

2.1 Observations from epidemiological studies

All of the studies used diagnostic criteria modified from those put forward by Masters *et al.*[38] On the whole neuropathologically confirmed cases were defined as "definite", and those with *characteristic* electroencephalographic appearances and appropriate clinical features were designated "probable". The analysis of electroencephalographic appearances is entirely subjective, and criteria such as "periodic synchronous discharges"[35] and "pseudoperiodic slow-wave spikes"[39] may not have been used consistently in different series. The clinical analysis presented by P. Brown based on his work in France[40] is notable for including neuropathologically confirmed cases of CJD only, and his recent study from the National Institutes of Health (NIH) looked at the clinical characteristics of 300 cases all of whom had been successfully transmitted to nonhuman primates.[41] Will and Matthews excluded four cases

of "possible" CJD (no typical EEG available) but included six cases of "possible" CJD of amyotrophic form (vide infra) in the analysis of clinical features in 158 cases.[42]

2.1.1 Familial versus sporadic

Masters' own series suggested that the proportion of cases that were familial (due to inherited factors) was 15%.[43] However, this value could be criticised as an over-estimate as cases in which a family member had already been investigated were more likely to be referred to this group. Indeed, the large scale epidemiological studies in England & Wales and France estimated the prevalence of familial CJD to be around 6%.[44,36] Interestingly, Galvez *et al* estimated the proportion of cases that were familial in their series from Chile to be 47%, a figure almost certainly related to the high prevalence of the mutation at codon 200 of *PRNP* in this population.[45] The estimate for familial CJD was generally accepted as between 5 and 10%, although with hindsight an accurate estimate could only be provided by the systematic screening of unselected cases of CJD for mutations of *PRNP*.

2.1.2 Age of onset, and disease duration

In Brown's 1986 study[40] the mean age of onset was 61.5 years (range 19 to 83), and the mean disease duration was 7.6 months (median 4.0). In his larger series of 1994,[41] in a subgroup of sporadic cases (n=234) the mean age of onset was 60 years (range 16 to 82, median 60) and the median disease duration was 4.5 months (range 1 to 130, mean 8). In the latter study, in the familial subgroup (n=40, with *PRNP* mutations demonstrated in 35) a younger age of onset and a longer duration of disease were noted. The longer durations of disease in younger patients noted in the earlier study may have partly been due to the same phenomenon.

Will and Matthews divided their patients into three groups: subacute, intermediate and amyotrophic.[42] The classification was based on the clinical courses of their patients, and while there was no difference in age at death of patients in the three groups, patients with intermediate and amyotrophic forms of CJD had longer courses. Intermediate cases were difficult to diagnose in life, as they masqueraded frequently as some of the commoner neurodegenerative diseases (such as Alzheimer's disease (ATD)). Numerically, however, these cases were small in number (12 out of 158 cases in their series).

2.1.3 Clinical courses

Cathala and Baron refer to a prodromal clinical stage in a third of patients, consisting of non-specific features such as fatigue, sleeping difficulties, weight loss, headaches, malaise and "sensations".[46] However, Knight argues persuasively that these symptoms are "...common, non-specific, and often noted later, when developments have lent them retrospective and possibly spurious significance."[47]

Symptoms and signs at the start of and during the course of CJD in the larger series are summarised in Table 3. The data available from Will and Matthews pertains to 137 definite and probable, subacute cases. With respect to the debut of their illnesses, 30-40% of patients had cognitive impairment alone, 30-40% had neurological disease in isolation and 20-30% had mixed features. As can be seen in Table 3, there is some variation in the reported frequency of features but this is probably due to differences in the classification of symptoms and signs in the various studies as well as chance variation of clinical features amongst the patients studied. Several general conclusions can nevertheless be drawn. Firstly, dementia was always present during the course of illness in these mostly sporadic CJD cases. Secondly,

myoclonus, which was rarely seen at the onset, was frequently noted during the course of disease. Thirdly, features of cerebellar, visual, pyramidal and extra-pyramidal dysfunction were noted regularly but were not universal. Finally, features of lower motor neurone dysfunction and convulsions were rare presenting features, and were unusual even during the course of illness.

2.1.4 Investigation results

Routine biochemical and haematological investigations were normal in these series, with the exception of liver function tests (32 abnormal out of 80 cases for whom results were available in Will and Matthews' study[42]). Computerised tomography (CT) was usually normal but occasionally revealed atrophy, especially in cases with more protracted illnesses. Examination of cerebrospinal fluid (CSF) was sometimes abnormal. Extrapolating from Will and Matthews' data, the CSF protein content was greater than 0.4 g/l in 45% of cases where it was examined.

Of the investigations performed, abnormalities in the EEG were the most useful in establishing the diagnosis. Given the inclusion of characteristic EEG appearances in the diagnostic criteria of these epidemiological studies, there would have been a tendency for over-ascertainment of this feature. Also, as already indicated, given the subjective interpretation of EEGs there may have been considerable variation in the types of abnormalities included. Will and Matthews found typical EEG findings in 84% of subacute cases, where an EEG had been performed. In their intermediate group (with atypical clinical courses) the EEG was typical in only 2/9 cases where it was performed. Brown *et al* report "periodic" EEG appearances in 60-80% of patients, and "triphasic 1 cycle/second" in 48-56%.

Systematic data on brain biopsy of suspected cases is available in Brown's NIH study. In 52 out of 55 autopsy-verified cases, the brain biopsy had revealed spongiform change. Against this high rate of diagnosis has to be considered the attendant risks of accidental transmission of CJD to other patients and staff.[48,49] The same study pointed out the utility of examining CSF by two-dimensional electrophoresis for a pair of novel proteins,[50,51] now identified as the 14-3-3 brain protein and detectable by immunoassay.[52] None of the studies give data on Magnetic Resonance Imaging (MRI) in cases of CJD.

In summary, these large epidemiological studies gave fairly consistent profiles of CJD, with respect to the proportion of cases that were familial, the patients' ages of disease onset and the durations of their disease, the clinical features of the illness, and the results of investigations. The major advances in the study of CJD in the 1980s arose from the field of molecular biology, in particular the experiments designed to test the "prion" theory of disease causation. Before embarking on a review of the theory and an appraisal of the key experiments, three unusual variants of CJD are discussed: those with long disease durations, and the so-called "amyotrophic" and "panencephalopathic" forms of CJD.

2.2 CJD of long duration

As noted, the duration of disease in sporadic cases of CJD is usually 4.0 to 4.5 months. In familial cases the illness is of longer duration, which may account partly for the longer course noted in younger patients (who are also more likely to have an inherited form of CJD). Will and Matthews give accounts of the clinical courses of their "intermediate" cases (6% of their

series), and three distinct types of disease progression are noted: a form of CJD characterised by slow but inexorable progression, a form in which a slow neurodegenerative process is followed by a rapid terminal phase, and (most rare of all) a rapid early course followed by a protracted terminal state in which there is little further decline.[42] As a group, these patients had a mean disease duration of 33.4 months. It is of note that 2 (of the 12) cases had family histories of neurodegenerative processes.

In a further seminal publication, P. Brown and colleagues summarised their experience with cases of CJD of disease durations longer than two years.[53] At the time of this study, the cases represented 9% of those with histopathologically verified CJD. They were characterised by a higher likelihood of having familial disease, a younger age of onset, and lower frequencies of myoclonus and periodic EEG appearances during the clinical illness. The difficulty of differentiating these cases from patients with other chronic dementing processes (particularly ATD) was stressed. Intriguingly, in transmission studies primate inoculations were less successful in these cases, than in those with shorter disease durations. (The incubation periods and disease durations in the inoculated animals did not differ from those inoculated with tissue from short duration cases.) In Brown's later NIH series, a lower rate of successful transmission was achieved with familial cases,[41] and the lower rate of successful transmission in their earlier series is probably due to the presence of cases of inherited CJD within the group of patients with long duration disease. This observation would also account for the unusual epidemiological characteristics of this group. Nevertheless, in the NIH series, there were still 9 (4%) sporadic patients with illnesses longer than two years, with the longest duration of disease being 4.5 years. Of the previously identified subtypes, there were three patients each in the slow, slow-fast and fast-slow groups.

2.3 Amyotrophic CJD

As indicated in the epidemiological studies, signs of lower motor neurone dysfunction are encountered in CJD, but these are uncommon and do not occur in the absence of more widespread cortical and cerebellar disease. Furthermore, their presence at the start of the clinical illness is extremely unusual. Salazar *et al* reviewed this subject in 1983, and came to the conclusion that "...the great majority of cases involving syndromes of dementia and early onset of LMN (lower motor neurone) signs are clinically and pathologically distinct from the typical cases of CJD and do not represent transmissible disease caused by unconventional viruses as presently understood."[54] They based their conclusion on the negative transmission studies they performed, and also on the atypical neuropathological appearances. The most consistent feature in the latter examinations was atrophy (with neuronal loss and gliosis) in the *frontotemporal cortex*. It is now recognised that frontotemporal atrophy is the pathological hallmark of the dementia that sometimes accompanies motor neurone disease.[55,56] Confusingly, there may be secondary spongiform change in these regions,[9] which may account for the previous inclusion of this disorder in the human spongiform encephalopathies. It is the present author's view that "amyotrophic CJD" is a misnomer, and in the majority of cases represents motor neurone disease with dementia. In Salazar *et al*'s series only 2 out of 33 cases with dementia and early lower motor neurone signs transmitted: both had chronic peripheral neuropathies and also typical CJD brain histology. They probably represented cases of CJD that had developed by chance in individuals with chronic neuropathies.

2.4 Panencephalopathic CJD

This pathological variant of CJD is described almost exclusively in the Japanese literature,[46] and is characterised by extensive *white matter* degeneration in cases of CJD. There is probably no difference in the clinical presentations of these patients, although reports have implicated a longer duration of disease, and evidence of cortical atrophy and white matter disease (on CT and MRI) in these cases.[57,58] A further report has implicated the coexistence of amyloid plaques.[59] The negligible experience of this entity in Western series makes it difficult to gauge its importance in CJD. It may represent a unique interaction of a strain (?) of CJD with the unusual host genotype of the Japanese population.[60]

3.1 The "prion" hypothesis

The suggestion that the agent of infectivity of scrapie may be devoid of nucleic acid was first made by Tikvah Alper and colleagues as far back as 1967.[212] They based this on the observation that scrapie infectivity was resistant to irradiation, including ultraviolet at 254 nm. Stanley Prusiner resurrected the concept in the early 1980s, and argued that the agent was composed mainly, if not wholly, of protein; hence the term *prion* (a *pro*teinaceous *in*fectious particle).[4] Prusiner's suggestion was met with considerable scepticism, but over the ensuing 20 years he performed a series of experiments to support his theory. He demonstrated that scrapie infectivity co-purified with a protein of 27-30 kDa molecular weight,[61,62] and that this infectivity was not dependent on polynucleotides.[63] The apotheosis of these experiments was the development of spontaneous neurodegeneration in transgenic mice carrying the murine equivalent of the codon 102 mutation in man.[64] Transmission of brain tissue from these animals into Syrian hamsters

and homotypic transgenic mice (but not Swiss mice) was successful.[65,66] In subsequent experiments PrP null mice were shown to be resistant to the intracerebral inoculation of scrapie,[67] and the "conversion" of soluble, protease-sensitive prion protein (PrP^{C}) to its protease-resistant, infective form (PrP^{Sc}) was achieved in a cell-free system.[68] Taken together, these experiments provide compelling evidence for the primacy of PrP in the pathogenesis of the spongiform encephalopathies. The interaction of PrP^{C} with PrP^{Sc} may enable the formation of more PrP^{Sc} (Figure 3). As well as mediating neurotoxicity, PrP^{Sc} may then convert more PrP^{C} to PrP^{Sc}. The theory is less effective in explaining the strain variability observed with scrapie, which is intuitively attributed to the agent including nucleic acids.[69] In order to accommodate the protein-only model and this observation, a "virino" model has been proposed. In this an essential but small nucleic acid component is protected by its close and integral association with PrP.[70] Alternatively, a "coprion" or "unified" theory postulates that although the agent itself is devoid of nucleic acid, it has the capacity to recruit cellular nucleic acid from the cell which it is infecting for replication.[71] Others, including Prusiner, have argued that the "variability" of the agent resides in glycoprotein modifications of the basic prion molecule,[72] or in conformational differences in the β-pleated quaternary structures of PrP^{Sc}.[73]

The strongest evidence to support the prion hypothesis has arisen from the study of inherited forms of CJD. To date, 22 separate mutations of the open reading frame (ORF) of the encoding gene of PrP have been linked with cases of inherited CJD (Table 4). Mutations have not been identified in normal controls,[75,95] and furthermore there have been no reported cases (so far) of inherited CJD worldwide not associated with any of the mutations identified.

The ease with which transmissibility is achieved according to this model is clearly dependent on the homotypic similarities between interacting prion proteins. It is suggested that the lower rate of successful transmission of inherited cases of CJD to experimental animals[41] is due to the difficulty PrP molecules derived from mutated alleles will have in interacting with "normal" PrP of the inoculated animal. Sporadic CJD is thought to arise from the chance conformational change of PrP^{C} to PrP^{Sc}. Some evidence for this is available from the observation that transgenic models in which prion protein is over-expressed have spontaneously developed transmissible spongiform encephalopathy.[96]

In a recent development on the prion theme the presence of a "protein X", which may act as a molecular chaperone in the formation of PrP^{Sc}, has been postulated.[97] The presence of this species-specific macromolecule may account for the need for ablation of the nascent PrP gene before transgenic mice expressing human PrP become susceptible to exogenous human prions.

3.2 *PRNP*, its rôle in iatrogenic and sporadic CJD, and further implications on the prion theory

The *PRNP* ORF has a site of common polymorphism at codon 129. At this site the amino acids methionine (Met) or valine (Val) are encoded. Homozygosity in general appears to confer increased susceptibility to the development of both iatrogenic and sporadic forms of CJD.[98,99] In iatrogenic disease, valine homozygosity appears to favour the development of CJD when the agent is introduced outside the central nervous system (such as in HGH recipients), whereas in cases of "central" inoculation (infected dura mater graft recipients and the like) methionine homozygosity is favourable.[100]

It is methionine homozygosity again that is strongly implicated in sporadic CJD, being identified in around 80% of cases in caucasian sporadic CJD series.[101,102] In one series, patients who encoded Met/Val at codon 129 had a median disease duration of 8.8 months, compared with median disease durations of 3.0 and 6.0 months respectively in Met/Met and Val/Val-encoded patients.[101] However, the number of patients carrying Val-encoding alleles was small in number (12 out of a total of 41 cases). It is also of note that in one large pedigree with inherited CJD, the age at death was influenced by this polymorphic site: carriers who were heterozygous at this site had a significantly greater age of death compared with those who were homozygous at this site (the mutation in this pedigree was always carried on Met-bearing alleles).[95] Once more these observations have been interpreted as being consistent with the prion theory, in that PrP molecules that are derived from *PRNP* genes that are homozygous at codon 129 will have an enhanced capacity for polymerisation, whereas PrP molecules derived from patients who are heterozygous at codon 129 will exhibit less efficient polymerisation. In the latter case, therefore, the disease will progress less rapidly, and patients will be afforded a degree of "protection" from disease development (hence their older age at death).

3.3 Familial CJD

In this section, the various forms of inherited CJD linked with mutations of *PRNP* are examined from a clinical perspective. Large scale population-based series in which mutations of *PRNP* were screened for, revealed that the true prevalence of familial CJD was between 11 and 13%.[101,102] This, it will be recalled, is roughly twice that estimated in epidemiological studies in

which a positive family history (of CJD) was the only criterion for consideration as a case of inherited CJD.

Although early descriptions attempted to correlate specific clinical and neuropathological phenotypes with particular *PRNP* mutations,[103] with greater experience it became clear that there was considerable variation especially in the clinical manifestations.[104,105] The attempt to analyse this phenotypic variability on the basis of the common polymorphism at codon 129 has yielded mixed results.[106,107]

3.3.1 Pro - Leu change at codon 102, and GSS

As described earlier, this was the first identified pathogenicity-associated *PRNP* point mutation. Although the initial publication claimed "linkage" with GSS (maximum lod score of 3.26),[25] it is now thought that the mutation itself is pathogenic.[108] It has been identified in a number of kindreds with familial CJD worldwide. It has also emerged that the clinical and pathological phenotypes associated with this mutation are highly variable. For example, in the German "Sch" pedigree, although the clinical illness was *broadly* consistent with GSS, specific clinical features varied significantly.[109] It was of note that one patient presented with anxiety and poor concentration, and others exhibited titubation and myoclonus during the clinical illness, all unusual features in GSS. Multicentric amyloid deposits were present in all autopsied cases, but there was a striking variation in the extent of spongiform change. The evaluation of the impact of other sites of genetic influence on this phenotypic variability is in progress, but, as mentioned, codon 129 has not been shown to influence phenotypic variability in an Italian pedigree.[107]

The final and most confusing issue with regard to GSS is the variety of *PRNP* mutations that are associated with this pathological phenotype. For example,

the point mutation at codon 117 was first described in association with GSS in Japan,[75] and the point mutations at codons 198 and 217 are associated with a form of GSS in which, in addition to multicentric plaques, there are neurofibrillary tangles ("the Indiana variant").[80,84] It should be noted that the codon 117 mutation has subsequently been described in association with a so-called "telencephalic" form of CJD.[76] In this form dementia is accompanied by pyramidal and extra-pyramidal features, and cerebellar signs are present to a variable extent.[110] The Indiana variant is not clinically distinct from GSS.[111]

In Japanese series, where the population prevalence of Val at codon 129 is low (Met:Val = 0.958:0.042 compared with Met:Val = 0.625:0.375 in caucasian British),[60] the presence of the triplet encoding Val is considered a pathogenic point mutation, and the associated disease dubbed GSS.[112] It is of note, however, that the amyloid plaques in this form of CJD are *unicentric* aggregations of prion protein, a form of prion protein deposition identified in sporadic cases in the West (and more often seen in association with alleles encoding Val at codon 129).[113,114] It appears therefore that the amino acid change Met to Val at codon 129 is not a "mutation" linked with GSS, but a polymorphism associated with plaque-like deposits of PrP on neuropathological examination. Curiously, like in GSS, ataxia tends to be a key clinical feature in these patients worldwide, and characteristic EEG appearances are encountered rarely.[115,113]

3.3.2 Glu - Lys change at codon 200

This mutation is of some historical interest, in that it appears to be the explanation for the high incidence (sometimes more than 100-fold) of CJD in certain ethnic groups. In the case of Libyan-born Jews, the high incidence of CJD was attributed to dietary factors, in particular the consumption of lightly

grilled sheep eyeballs.[116] It is now known that clinical illness in this population cosegregates with the codon 200 mutation.[117] The mutation has been identified in CJD clusters in Slovakia and Chile,[118,119] and in ethnic British, French and Japanese patients.[120,121,122] The classical disease phenotype associated with this mutation resembles sporadic CJD. As in sporadic CJD characteristic EEG appearances are commonly encountered, and on neuropathological examination typical spongiform encephalopathy is present. Transmissibility is more readily achieved than with other forms of familial CJD.[41] Despite the superficial similarities in clinical, neuropathological and transmission characteristics, a detailed study of a large Libyan Jewish pedigree revealed considerable heterogeneity of clinical features, including rarities (for CJD) such as demyelinating polyneuropathy.[104,123] Another affected member of the same pedigree with clinical and pathological features of fatal insomnia (vide infra) has been reported.[124] There is some debate on the degree of penetrance of this mutation. Initially thought to 0.56 penetrant,[125] it is now argued that it is (like all other *PRNP* mutations) fully penetrant *if the carrier lives sufficiently long.*[126]

Other *PRNP* mutations with clinical phenotypes resembling sporadic CJD include the Val - Ile change at codon 210[83] and the Asp - Asn change at codon 178 (in association with Val at codon 129 of the mutation-bearing allele).[78] The latter mutation is not, however, associated with characteristic EEG appearances.[103] It also emerges, fortuitously, as the first clinically described pathogenicity-associated *PRNP* mutation: an identical mutation was demonstrated[127] in DNA extracted from archival material on the first case of familial CJD ("Paul Backer"), described by Kirschbaum in 1924.[128]

3.3.3 Fatal Familial Insomnia (FFI)

The syndrome was first described in 1986 by Lugaresi *et al.*[129] A 53-year old man presented with progressive insomnia and dysautonomia. A previously sound sleeper, he could only sleep for two to three hours at night. He became impotent and had loss of libido. There was episodic salivation, lacrimation and rhinorrhoea, and he exhibited orthostatic diaphoresis, pyrexia, difficulties with micturition and constipation. Two months later, he could sleep for only an hour each night, and he was frequently disturbed by vivid dreams. He developed progressive dysarthria, intention tremor of his limbs and gait ataxia. Examination at this stage revealed miosis, cerebellar signs and brisk tendon reflexes. He was noted to lapse into a stuporose state if left alone, in which he performed complex and apparently purposeful gesturing and breathed noisily. He could be awakened quickly by light stimuli. As his condition progressed, there were oculomotor disturbances (limitation of upward gaze and saccadic movements), myoclonus, and irregular breathing patterns. Terminally he became confused and disorientated, had episodes of motor agitation, and exhibited severe truncal dystonias. The total duration of his illness was nine months.

Routine laboratory investigations were normal, with the exception of mildly elevated CSF protein (0.76 g/l) and elevated urinary catecholamines. EEG was not characteristic of CJD, but revealed diffuse slow waves and latterly became isoelectric. His dreamlike states coincided with EEG desynchronisation, but physiological EEG patterns of sleep were absent. A pharmacological response to a short-acting benzodiazepine antagonist could be demonstrated both clinically and electrophysiologically.

Examination of his pedigree revealed at least four other affected family members, including two sisters of the propositus.

Neuropathological examination revealed severe cell loss and reactive astrocytosis of the anterior and dorsomedial nuclei of the thalamus. There was some astrogliosis of the olivary nuclei in addition, but the neocortex and white matter were entirely normal with *no evidence of spongiform encephalopathy*. Similar neuropathological findings were found when tissue from one of the sisters of the propositus was re-examined.

The causation of this unique inherited neurodegenerative process remained unknown until 1992, and the identification of the codon 178 (Asp - Asn) *PRNP* mutation in affected members of this pedigree.[7] By then the family had been studied in greater detail, and it had become apparent that in addition to insomnia and dysautonomia, dysarthria, ataxia, myoclonus and pyramidal signs were invariably present in affected members. Memory and attention deficits were minimal in the early stages, but tended to progress with time. During "sleep" there was loss of the slow-wave and rapid-eye-movement phases. The mean age at onset was 49 years, and the mean duration was 13 months.

More extensive neuropathological evaluation revealed various degrees of atrophy and reactive astrogliosis in the cerebral and cerebellar cortices, in addition to the selective thalamic and olivary degeneration noted before. Spongiosis of the cerebral cortex was noted in only one out of the seven cases examined.

Although readily accepted as a form of inherited CJD, it was not known how an identical *PRNP* mutation (Asp - Asn at codon 178) could produce such diverse clinical and neuropathological phenotypes. (It will be recalled that the mutation was first described in association with inherited CJD which was phenotypically similar to sporadic CJD.) This issue was elegantly resolved

later in 1992, when the common polymorphism at codon 129 was found to determine the disease phenotype: when the allele bearing this mutation encoded Met at codon 129 FFI was seen, whereas in association with Val CJD was observed.[106] There appear to be distinct differences between the conformational structures of the respective mutant PrP molecules.[130] Although initial attempts at transmission of FFI were unsuccessful,[41] this has recently been successfully carried out to laboratory mice[131] and transgenic mice expressing human PrP.[132]

3.3.4 "CJD" related to extra repeat insertions

The region between codons 51 and 91 of the *PRNP* ORF normally comprises one nonapeptide and four octapeptide repeats.[133] Insertions of two and between four and nine extra octapeptide repeats have been associated with CJD.[85,86,87,89,94] Phenotypically, these cases appear to resemble CJD, with two notable exceptions. Firstly, in the large pedigree reported from the South East of England with a 144 base pair insertion (six extra repeats), there was striking diversity in the clinical phenotypes of affected cases.[89] Diagnoses such as General Paralysis of the Insane, "spinal sclerosis", "cerebral softening", cerebral thrombosis, dementia praecox, Parkinsonism, Huntington's disease, Pick's disease and ATD had been attached to affected members of the pedigree through the ages. This was accompanied by considerable variation in the neuropathological features of cases. At one extreme the changes of CJD were so typical that sections were used to illustrate spongiform encephalopathy in Greenfield's textbook of neuropathology, and at the other extreme no abnormalities were present except on immunocytochemistry.[134,135] The final point of note in this family was that the nucleotide sequence of the mutated allele had remained stable over at least six generations.[95] This contrasts with neurodegenerative processes associated with trinucleotide repeat sequences, where there are

expansions of repeat sequences in successive generations.[136] This molecular biological mechanism appears to correlate with the clinical phenomenon of anticipation, where carriers in successive generations are affected at an earlier age (and more severely). Anticipation is not a feature of inherited CJD.

Secondly, in their report on a case of dementia associated with a 216 base pair insertion (nine extra repeats), Duchen *et al* argued that the presence of neuritic plaques staining positively for β-amyloid protein and tau protein indicated that this case represented a transition (neuropathologically, at least) between CJD and ATD.[94] From the clinical perspective, however, this patient's illness was entirely compatible with familial CJD.

3.3.5 Pro - Leu change at codon 105, and the amber mutation at codon 145

These mutations were described in familial cases of CJD in Japan. Almost uniquely for cases of CJD (sporadic or familial) patients carrying the mutation at codon 105 presented with spastic paraparesis.[74] Dementia eventually supervened, but there were no cerebellar features or myoclonic jerks. Characteristic EEG appearances were not seen. Neuropathological examination revealed amyloid plaques (hence the designation "GSS"), and vacuolation with loss of myelin of the pyramidal tracts in the brainstem and spinal cord. As indicated, spinal cord features are rare in CJD both clinically and pathologically, although recently PrP has been detected by immunocytochemistry in the spinal cords of sporadic and HGH-associated cases.[137]

The mutation at codon 145 leads to the generation of a "stop" codon, and was clinically associated (in a single patient) with a slowly progressive dementia (greater than 10 years' duration).[77] Neuropathology revealed no spongiform change, but there were anti-PrP positive amyloid plaques

(negative with anti-βA4 antibody), hence its designation as "GSS". Elegant immunocytochemical studies have shown that the amyloid plaques in this case contain only the N-terminal of PrP.[138] If the prion hypothesis is accepted, the implication of this observation is that the polymerisation of PrP molecules in this setting is limited to mutant molecules. This is analagous to the transmission characteristics of tissue from the murine GSS model, where successful transmission was achieved only where the recipient also carried the codon 101 mutation. Presumably the interaction requires recognition of *homologous* PrP molecules.

In the foregoing descriptions, the spectrum of clinical and pathological phenotypes associated with *PRNP* mutations have been explored. While providing an invaluable tool for the investigation of familial neurodegeneration in general and familial CJD in particular, there are dangers in attributing rigid clinical definitions to individual mutations. Not only is this over-simplistic, but such attempts may ignore the influence of modifying genes on *PRNP*. As noted in the case of the common polymorphism at codon 129, modifying mechanisms may even be contained within the same ORF.

The apolipoprotein E (APOE) gene has emerged as another possible disease-modifier. Following the observation of this gene's influence on ATD,[139] cortical Lewy body disease[140] and Down's syndrome,[141] studies are in progress to examine the influence of the APOE gene on CJD. In a French study, the ε4 allele of the APOE gene was found to be a risk factor for the development of CJD, and a subgroup analysis of 14 patients (out of a total of 61) bearing the ε2 allele revealed that these patients had a longer disease duration.[142] The study has subsequently been criticised for its inappropriate selection of controls,[143] and in the hands of a second (German) group APOE

gene status did not influence the development or outcome of CJD.[144] Apolipoprotein E in the CSF was also examined, with normal results.[144] In the study by Pickering-Brown *et al* in which 8 out of 20 patients had amyloid plaques, the presence of the ε2 allele was associated with a later age of onset.[114] It is conceivable that the influence of the APOE gene in CJD is predominantly on the subset of patients who manifest amyloid plaques. This is biologically plausible, in that APOE is a constituent of PrP amyloid plaques.

It is finally worth considering the normal function of PrP. PrP null mice were found to develop normally and have normal gross behavioural characteristics.[145] This was a surprising finding given the high conservation of PrP among mamalian species and its wide expression in early embyogenesis. Recently it has been demonstrated that PrP null mice exhibit weakened γ-amminobutyric acid type A receptor-mediated fast inhibition and long-term potentiation.[146] The relevance of this observation to the underlying pathogenic processes in CJD is unclear at present. Of more obvious relevance (in view of the clinical observations in FFI) is the recent description of altered circadian activity rhythms and sleep patterns in these mice.[147]

4.1 Iatrogenic CJD

CJD acquired by iatrogenic mechanisms is extremely rare, with the total number of cases *worldwide* currently numbering less than 120. Nevertheless, in addition to the public health implications, these cases are important for the insights they have given into the mechanisms by which spongiform encephalopathies develop and propagate. Iatrogenic CJD has been shown to arise from five main sources:

4.1.1 Human pituitary-derived hormones

Since the first descriptions of CJD in recipients of HGH in 1985,[148,3] more than 75 cases have been identified. In addition, there have been at least two cases associated with gonadotrophin (HGnH) therapy.[149] Up until 1985 these hormones were derived from human cadavers, and, for example in the case of HGnH, up to 800 cadaveric pituitary glands pooled in a single batch of treatment.[150] The outbreak of cases in the US, Britain and France implies that multiple independent contaminations took place.

In contrast to patients with sporadic and familial CJD, the clinical illness in these patients is strikingly homogeneous. The disease is characterised by a progressive cerebellar syndrome, with little or no cognitive impairment at the start and the absence of typical EEG appearances.[151] For example, in their clinical descriptions of 1985, the physicians from San Francisco and Bethesda both highlight the insidious development and relentless progression of truncal and limb ataxia in their patients.[148,3] Both patients exhibited personality change at the start of their illnesses, in particular appearing apathetic and unconcerned about their predicament. As the disease progressed titubation, limb rigidity, myoclonus (including startle responses) and bulbar dysfunction supervened. Terminally, the patients were probably demented, and were akinetic and mute. In some respects the clinical courses of these patients were similar to those described in kuru, particularly in children (vide supra). This similarity has led to the suggestion that it is the peripheral (outside the central nervous system) route of inoculation of the CJD agent that leads to this clinical phenotype.

The putative "incubation period" after agent inoculation and disease development is difficult to estimate, as HGH therapy was received as injections twice or three times per week over many years. However, based on

the mid-points of the respective courses of treatment, an estimate of 15 years with a maximum of 30 years in a single case has been made.[8] The durations of clinical illness in the first described cases were 6 and around 18 months.[148,3]

The higher prevalence of valine homozygosity at codon 129 in patients with iatrogenic CJD after the peripheral inoculation of agent has been mentioned previously. The reason for this, or indeed why methionine homozygosity should be favoured when the agent is inoculated centrally, is unclear.

4.1.2 Human cadaveric dura mater grafts

These grafts which were used in neurosurgery to repair dural defects, have been implicated in at least 25 cases of CJD in graft recipients.[152] The median incubation period has been estimated to be around 67 months,[48] and their clinical illnesses on the whole have resembled sporadic CJD. The recent clinical descriptions of four of these patients are notable.[153] In all of them the "Lyodura" grafts had been placed in the posterior fossa and they all presented with cerebellar features (all four were ataxic at presentation, and three had dysarthria). The cerebellar presentations in these patients may have been related to the inoculation of agent into or near the cerebellum, or due to the "decompensation" of function manifesting first at this site (two of the patients had had cerebellar astrocytomas resected, and two had had posterior fossa decompressions for Arnold-Chiari malformations). The evolution of illness in all four, however, was quite distinct from that seen HGH-related CJD cases, and one of the patients developed periodic triphasic complexes on the EEG.

4.1.3 Corneal transplantation

The first clinical description (in 1974) of person to person transmission of CJD was following a corneal transplant.[154] The donor and recipient had clinical illnesses typical of CJD, and the diagnosis was confirmed pathologically in both. Subsequent experimental studies have confirmed the infectivity of corneas of animals inoculated with the CJD agent,[155] and its spread along visual pathways after intraocular inoculation.[156] It is of note that the recipient (who developed her illness 18 months after the procedure) had lethargy, nausea and ataxia as her initial symptoms, and developed dysphagia, myoclonus and brisk reflexes as the disease progressed. Terminally she was mute and had decorticate posturing. Despite the experimental studies referred to, cortical blindness was not a key feature.

4.1.4 "Contaminated" EEG depth electrodes and operating instruments

In 1977 two cases of CJD in individuals who had had stereotactic EEG recordings were reported.[157] The victims were aged 19 and 25, and it seemed unlikely that they could both have developed sporadic CJD by chance. Two of the electrodes used in both procedures had been used previously in a patient with a four-month history of dementia, ataxia, involuntary movements, myoclonic jerks and akinetic mutism, in whom CJD was confirmed (subsequently) at autopsy. The heat sensitvity of the electrodes had prevented their autoclaving, and they had been "sterilised" with 70% alcohol and formaldehyde vapour. One of the electrodes has subsequently transmitted CJD to a chimpanzee 18 months after intracerebral implantation.[158]

Finally, a total of four cases in which CJD was probably acquired at the time of neurosurgery have been reported.[159,160] The three cases reported by Will

and Matthews were of note for their inclusion in the descriptions of "subacute vascular encephalopathy" by Nevin *et al.*

Iatrogenic cases of CJD, although insignificant in terms of numbers, have contributed to the study of this enigmatic illness. The variable clinical phenotypes associated with the different routes of inoculation is a particularly pertinent observation. It is argued that with *any* exogenous (outside the central nervous system) source of infectivity, the clinical phenotype will resemble that in HGH recipients (with progressive cerebellar ataxia).[161] These speculations were lent enormous significance following the recent description of a novel form of CJD in the UK.

5.1 New variant Creutzfeldt-Jakob disease (vCJD)

The description by Will and colleagues of a form of CJD with a unique and hitherto unrecognised pathological phenotype probably extends the spectrum of human spongiform encephalopathy.[162] The original ten cases were identified between March, 1995 and January, 1996 at the unit where the current study was based. The cases were characterised clinically by an unusually young age of onset for sporadic CJD (mean age at onset = 26.3, median = 28, range = 16-39; n = 11 (data including a further pathologically confirmed vCJD case from France[163]), a relatively long disease duration (mean = 7 months, median = 13, range = 7.5-23), unusual presenting features for CJD (such as sensory disturbance and "pure" psychiatric features), atypical EEG findings, and absence of pathogenicity-associated *PRNP* mutations and Met homozygosity at codon 129. Cerebellar ataxia was a prominent feature in the majority of the patients' illnesses. The unifying pathological feature in all cases was the widespread presence of prominent

PrP plaques (termed "florid" plaques), which stained densely with anti-PrP antibody. Attempts to identify similar pathology in central nervous system tissue from previously diagnosed (including young) cases of CJD, in the UK and elsewhere, has been unsuccessful.

The finding has fuelled the search for pre-mortem diagnostic tests, especially where vCJD is suspected in young individuals. Even by immunoassay the brain 14-3-3 protein in CSF appears to lack specificity in suspect CJD cases.[164] Meanwhile, Collinge and colleagues have demonstrated that the physiochemical characteristics on Western blotting of PrP^{Sc} derived from patients with vCJD are distinct from those of patients with sporadic CJD and iatrogenic CJD related to HGH.[165] The application of this technique to study PrP^{Sc} in tonsil tissue (successfully, in a single vCJD case to-date) is a promising development.[166]

In this introduction the early clinical descriptions of CJD have been reviewed. The clinical data from large-scale epidemiological studies of this disorder have then been summarised. The "prion" hypothesis has been described, and the rôle of *PRNP* in sporadic, familial and iatrogenic CJD examined. The spectrum of disorders associated with mutations of *PRNP* has been described. The poor correlation between specific *PRNP* mutations and clinical phenotypes has been noted. Furthemore, the association of *PRNP* mutations with cases of familial CJD has led to the expansion of the clinical boundaries of this disorder. The clinical presentations of the small number of cases of iatrogenic CJD have been reviewed, and particular note made of the phenotypic differences between cases in which inoculation occurs inside and outside the central nervous system. Finally, reference has been made to vCJD.

Inevitably, a completely comprehensive review of the clinical manifestations of CJD is impossible. For example, cases presenting acutely (resembling strokes)[167] and those found in association with ATD[168] have not been discussed. All researchers of the disorder are aware of the immense diversity of clinical features, and the medical literature is replete with descriptions of unusual presentations of CJD.This study is one means of identifying exactly how common these unusual phenotypes of CJD are. Also it can be ascertained whether the phenotypic expressions of the patients in this series vary significantly from those included in previous descriptions. Speciation along clinical grounds, if feasible, will enable correlative studies between clinical phenotypes on the one hand and neurpathological and molecular biological characteristics on the other. Systematic data on CJD in the UK between 1990 and 1994 will also act as a useful background against which probable novel forms of human spongiform encephalopathy, such as vCJD, can be studied.

METHODS

1.1 Clinical methodology

All patients in the study were referred to the U.K. national surveillance unit, as suspected cases of CJD. The study design has been reviewed,[169] and has been adopted in the Biomed I programme for the surveillance of CJD in the European Community.[170] In order to ensure comprehensive coverage and avoidance of bias, a variety of sources are requested to refer cases. Briefly, suspected cases of CJD were referred by Neurologists, Physicians and Psychiatrists during the terminal illness. Cases manifesting typical EEG appearances were referred by Neurophysiologists. The patients were visited by a neurologist (RGW, TFGE, RDS or MZ) attached to the unit, and data on the evolution of the illness was collected by interview of a close relative or friend, and perusal of clinical records. The patient was examined, and results of investigations collected from the case sheet. Where possible electrophysiological and imaging studies were reviewed personally. Blood was collected from patients for storage and extraction of DNA for *PRNP* genome analysis. The latter was performed after appropriate counselling of relatives, in line with guidelines.[171] Post mortem confirmation is sought in all cases, and this is achieved in almost 70% of cases referred to the unit.[172] Neuropathology colleagues are another source of referral. Cases that were found to have CJD at post mortem were referred to the unit, and clinical information collected from interview of relatives and examination of records retrospectively. The Office of Population, Census and Statistics acts as a final check, and all death certificates carrying rubrics suggestive of CJD were screened and forwarded to the unit. The case records of these cases were examined, and if the diagnosis of CJD could be sustained clinical information was collected retrospectively again.

The study is part of the effort to detect any influence of the epidemic of BSE on the incidence and characteristics of CJD. Therefore, in addition to

gathering clinical data, a detailed questionnaire on patients' lifestyles was completed. In this, information on family history, occupation, animal exposure, diet and residence was collected from cases and age and sex-matched controls. Results of this on-going epidemiological study are published annually.[172,161,173]

The cases were classified according to standardised criteria, modified from Masters *et al.*[38] Definite cases have been confirmed pathologically, either by examination of the whole brain after autopsy or following brain biopsy. Probable cases have not been confirmed pathologically, but have characteristic EEG tracings (periodic sharp wave complexes throughout the recording) in an appropriate clinical context. The latter is defined as a rapidly progressive dementia in a patient with at least two of the following clinical features: myoclonus, cortical blindness, pyramidal/extrapyramidal/cerebellar dysfunction, akinetic mutism, and early neurogenic muscle atrophy. Possible cases have not been confirmed pathologically and do not manifest typical EEG appearances, but they have had a rapidly progressive dementia with at least three of the clinical features listed. At post mortem, patients suspected of having CJD may turn out to have succumbed to another neurodegenerative process: these patients have been classified as "other" in the current study. A further group of patients (other#) who at the time of referral did not fulfil criteria for inclusion as either a probable or possible case, and for whom a final tissue diagnosis was unavailable (usually because of recovery or lack of progression of the clinical illness) was identified, for comparison with the patients designated other.

The patients in this study were referred consecutively to the unit between May, 1990 and the end of April, 1994 (representing the first four years of the surveillance project). The patients seen by each neurologist have been listed

in Appendix 1. The clinical information was compiled at the time of the visit in a standardised pro-forma, and for the purposes of this study clinical data from *all* definite, probable, other and other# cases identified during this period was extracted by RDS. The sign(s) at presentation, the clinical characteristics during the early phase, and during the course of the illness were listed separately (Appendix 2). The presence or absence of specific features was recorded as a binomial variable (presence = "1", absence = "0"). Other clinical variables such as age at onset (which was estimated as accurately as possible from the clinical enquiries) and disease duration were listed as continuous variables in a database created on the C-Stat for Windows package. Other information included in the database were: class ("1" = definite, "2" = probable, "4" = other),* type ("1" = sporadic CJD, "2" = familial CJD, "3" = iatrogenic CJD), presence/absence of *PRNP* mutation, genotype at codon 129, and EEG/liver enzyme/CSF/neuroimaging data ("1" = abnormal, "2" = normal). The CJD reference number (the patient identification number in use at the unit), and the unit neuropathology reference number (in those cases where the neuropathological examination had been performed in Edinburgh) were also listed. In familial cases, further clinical information particularly pertaining to family history was gathered directly from the records. In the process of collecting the data, particularly interesting investigation results were highlighted, so that these could be discussed later in the study in greater detail.

Comparison of ages of onset and disease durations between the different types of CJD were carried out using Mann-Whitney U tests. Analysis of clinical characteristics at different stages of the illness was performed separately in sporadic, familial and iatrogenic cases. Using this data an

* Class 3 or "possible" cases of CJD were not considered further in this study. An explanation for this omission is given, and the possible consequences explored, in the discussion section (page 68).

attempt was made to identify particular CJD phenotypes, for example Heidenhain's variant, CJD with progressive cerebellar ataxia, and CJD of long duration. Comparison of clinical characteristics *between* different forms of CJD was performed using Spearman rank correlation. The clinical features during the course of illness in definite and probable cases were compared with those in cases classified as other using standard tests of sensitivity and specificity. Of course, at the time a patient is referred to the unit, their final outcome (in terms of death, stability or recovery) is unknown. In order to validate the sensitivities and specificities of the clinical features, in separating cases of CJD from those that are not *at the time the signs are elicited*, a further comparison was undertaken between other and other# cases.

2.1 Neuropathology

Neuropathological examinations were sometimes performed at other centres. The following protocol was used for the examination of tissue at the surveillance unit. After fixation of the brain in 10% formalin, tissue blocks were obtained from all cortical areas, the basal ganglia, thalamus, hypothalamus, cerebellum and brainstem. The blocks were decontaminated in 96% formic acid before routine processing into paraffin wax. Five μm sections were cut and stained by conventional histological techniques and by immunocytochemistry for prion protein using a polyclonal antibody raised against scrapie-associated fibrils (courtesy of Dr. J. Hope, MRC/AFRC Neuropathogenesis Unit, Edinburgh). Histological examination was kindly performed by Drs. Jeanne Bell and James W. Ironside.

In correlating clinical features with sponge distribution, the clinical data from individual cases was condensed and the area(s) of brain most likely to be

impaired, based on conventional knowledge of function localisation, was(were) identified. The corresponding histological report was perused blindly, and areas of brain showing spongiform change identified, based on the description of sponge distribution in the report. The degree of consistency between reports was inadequate to allow quantification of spongiform change. Clincal and pathological correlation for each area of brain was performed by comparing (in 2 X 2 tables) the frequency with which clinical features and spongiform change co-existed. Cases showing anti-PrP positive plaques were identified for correlation with clinical and molecular biological features.

3.1 Molecular biology

Genomic DNA was extracted using standard procedures, and the single *PRNP* exon encoding the entire ORF was amplified by polymerase chain reaction (PCR). The amplification reaction (in a final volume of 50 µl) contained 100 ng of the genomic DNA, 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 1.5 mM $MgCl_2$, 0.2 mM of each deoxynucleotide triphosphate, 1 µM of each oligonucleotide primer and 2.5 units of Amplitaq (Perkin Elmer Cetus). The sequence of the oligonucleotide primers were 5'-CGCAAGCTTGAACTCTGACATTCTCCTCTTC-3' (primer A) and 5'-TTCGAATTCCTCCCTCAAGCTGGAAAAAG-3' (primer B), which are situated 5' and 3' to the human *PRNP* ORF respectively. The mixture was overlaid with 100 µl mineral oil and subjected to 35 temperature cycles (1' 95°C, 1' 50°C, 1' 72°C) in an Omnigene thermal cycler. To analyse and purify the PCR products, the reaction was loaded on a 1.5% agarose gel, electrophoretically separated and stained with ethidium bromide. The amplified *PRNP* ORF was eluted according to standard procedures and subjected to (.1) single-strand

conformational polymorphism (SSCP) analysis,[174] (.2) direct sequencing or sequence analysis following cloning and (.3) a modified direct sequencing protocol[175] for fast screening of several known mutations and the common polymorphism at codon 129.

3.1.1 To detect mutations, SSCP analysis was performed. 5 ng of the amplified, eluted *PRNP* ORF was reamplified with each of the following pairs of oligonucleotide primers to generate a set of four overlapping products covering the whole ORF:

Pair 1: 5'-CTGACATTCTCCTCTTC-3' (primer C) and
5'-TTGTTCCACTGACTGTG-3' (primer D);

Pair 2: 5'-GCCCTGGAGGCAACCGC-3' (primer E) and
5'-GTAGCCGCCAAGGCCCC-3' (primer F);

Pair 3: 5'-TGGCACCCACAGTCAGT-3' (primer G) and
5'-TTCTCCCCCTTGGTGGT-3' (primer H);

Pair 4: 5'-CGTGAAAACATGCACCG-3' (primer I) and
5'-CCTCAAGCTGGAAAAAG-3' (primer K).

Apart from the choice of primers, the PCR conditions were different to the first round of amplification in containing 2.5 µCi of (α -^{32}P)-dCTP (Amersham) with cycling conditions of 0.5' 95°C, 1' 40°C, 2' 72°C. 2 µl of the reaction was mixed with 9 µl of stop solution (95% formamide and 10 mM NaOH), heated to 95°C for 2', flash-cooled on ice and 2 µl of this sample loaded on to a Hydro-Link^{TM} gel (AT Biochem), that was prepared and run according to the manufacturer's recommendations. The radioactively labelled products were detected by autoradiography and mutations in specific samples were revealed in comparison with normal *PRNP* ORF by changes in the migration pattern due to point mutations, deletions or insertions.

3.1.2 The exact nature of the changes in SSCP analysis was determined either by direct sequencing of the the amplified and gel-purified *PRNP* ORF with Circum Vent^{TM} polymerase (New England Biolabs, according to the manufacturer's recommendations) or by sequencing with Sequenase Version 2.0 T7 DNA Polymerase (USB) following cloning in pBluescript IIKS(-) (Stratgene). The cloning was done by standard procedures making use of the HindIII and the EcoR1 site in the primers A and B, respectively. To exclude PCR artefacts the products of three independent PCR reactions were analysed in parallel. The sequencing primers were the synthetic oligonucleotides used in the SSCP analysis (primers C to K).

3.1.3 Fast screening for the known mutations at codon 102, 178 and 200 and for the common polymorphism at codon 129 was done by a modification of the direct sequencing protocol. The synthetic oligonucleotides primer G, 5'-CTGCTCATGGCACTTCC-3' (primer L), 5'-GATTGTGATATTGACGC-3' (primer M) or 5'-CAAGGGGGAGAACTTCA-3' (primer N) were used for revealing the genotype at codon 102, 129, 178 or 200 respectively, and were radioactively labelled at their 5' end with (γ -^{32}P)-ATP and T4 polynucleotide kinase according to standard procedures. 1.2 pmole of the labelled primer was mixed with 10 ng amplified, gel-purified *PRNP* ORF and 2 units of Vent$_R$^{TM}(exo-) DNA polymerase (New England Biolabs) in 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 20 mM Tris-HCl (pH 8.8), 5 mM $MgSO_4$ and 0.2% Triton X-100 made up to a final volume of 15 µl. 3.2 µl of this mixture was added to 3 µl of a nucleotide mix, containing either 100 µM dATP, 100 µM dCTP, 100 µM dGTP and 720 µM ddTTP (in the case of primers G, L and M) or 100 µM dATP, 100 µM dCTP, 100 µM dTTP and 720 µM ddGTP (in the case of

primer N). The reaction was then overlaid with 100 µl mineral oil and the primer was elongated and simultaneously terminated at the polymorphic position by subjecting it to 25 cycles of PCR (20" 95°C, 20" 37°C, 20" 72°C). After adding 4 µl stop solution, 2 µl of the reaction were loaded on a 10% 7M urea polyacrylamide gel. Depending on the homozygous or heterozygous genotype of the patient, one or two products were created, which were separated by electrophoresis and visualised by autoradiography.

The techniques were developed by Drs. Otto Windl and Peter Estibeiro, and Prof. Rick Lathe at the AFRC Centre for Genome Research, University of Edinburgh. TFGE and the author extracted DNA from all the analysed cases, and the specialised molecular biological techniques were performed by Ms. Maureen Dempster and other scientists at the AFRC Centre for Genome Research. The author assisted the scientists in their laboratory on a number of occasions, discussed the techniques with them and participated in the data analysis.

Twenty-six patients in this study had their molecular biological examinations performed at the Prion Disease Group, at St. Mary's Hospital, London (Appendix 3). Their methodology was as follows.

The *PRNP* open reading frame (ORF) was PCR amplified from 1 µg genomic DNA. The reaction included 25 pmol oligonucleotide primer, 50 mM Kcl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 0.01% gelatin, 200 mM each

deoxynucleoside triphosphate, 5% DMSO and 2.5 units Taq polymerase [Amplitaq, Perkin Elmer Cetus] in a total volume 100 µl; cycling was: 94°C, 5 min; 35 cycles of [94°C, 30s; 57°C, 30s; 72°C, 60s]; 72°C, 5 min in a Techne PHC-3 thermal cycler. Synthetic primers for amplification were:

Oligo 3: 5'-GTGGCCACATGGAGTGACCTGGGCCTC-3'

Oligo 2: 5'-GAAAGATGGTGAAAACAGGAAGACC-3'

(for mutation analysis)

Oligo 5: 5'-GACCTGGGCCTCTGCAAGAAGCGC-3'

Oligo 6: 5'-GGCACTTCCCAGCATGTAGCCG-3'

(for detection of insertions and deletions).

10 µl of product (oligos 5 and 6) were resolved by gel electrophoresis (1% agarose); band sizes were compared with controls (normal alleles, alleles with insertions or deletions in the octapeptide repeat region). Products amplified with oligos 2 and 3 were first quantitated (agarose gel electrophoresis); equal loadings were selected. In general 10 µl was denatured (40 µl NaOH, 25 mM EDTA, 5 min), neutralised (50 µl 0.6 M Tris-HCl [pH 7.4], 1.5 M NaCl), and vacuum blotted to nitocellulose (Hybond-N, Amersham). After baking, filters were prehybridised in 6 x SSPE (0.9 M NaCl, 60 mM NaH_2PO_4, 6 mM EDTA; pH 7.4), 0.1% BLOTTO (fat free powdered milk), 0.1% SDS, 1 mM EDTA, 100 µg/ml denatured salmon sperm DNA, 36°C, 3h. Filters were hybridised for 16h under the same conditions in the presence of 10^6 dpm/ml [^{32}P] end-labelled oligonucleotide, one pair for each as listed below. Filters were washed (3 M tetramethylammonium chloride, 50 mM Tris-HCl [pH 8.0], 2 mM EDTA and 0.1% SDS, 36°C, 15 min; followed by

two further washes at 47°C, 15 min) prior to autoradiography for 4h. Allele-specific hybridisation with the following oligonucleotides was performed to detect all known mutations and polymorphisms of *PRNP*:

Oligo 13: 5'-CTTCACCAAGACCGA-3' (LYS 200)

Oligo 14: 5'-CTTCACCGAGACCGA-3' (GLU 200)

Oligo 15: 5'-AACAAGCCGACTAAG-3' (PRO 102)

Oligo 16: 5'-AACAAGCTGAGTAAG-3' (LEU 102)

Oligo 17: 5'-CGGCTACATGCTGGG-3' (MET 129)

Oligo 18: 5'-CGGCTACGTGCTGGG-3' (VAL 129)

Oligo 26: 5'-GCTGCAGCAGCTGGG-3' (ALA 117 PvuII+)

Oligo 27: 5'-GCTGCAGCGGCTGGG-3' (ALA 117 PvuII-)

Oligo 28: 5'-GCTGCAGTGGCTGGG-3' (VAL 117 PvuII-)

Oligo 25: 5'-GCTGCAGTAGCTGGG-3' (VAL 117 theoretical)

Oligo 31: 5'-GAGAACTCCACCGAG-3' (PHE 198)

Oligo 32: 5'-GAGAACTCCACCGAG-3' (SER 198)

Oligo 36: 5'-ATCACCCGGTACGAG-3' (ARG 217)

Oligo 37: 5'-ATCACCCAGTACGAG-3' (GLN 217)

Oligo 38: 5'-GGGGCTTGGCGGCTA-3' (GLY 124: polymorphism GGG)

Oligo 39: 5'-GGGGCCTGGCGGCTA-3' (GLY 124: polymorphism GGC)

Oligo 47: 5'-AGTAAGCCAAAAACC-3' (PRO 105)

Oligo 48: 5'-AGTAAGCTAAAAACC-3' (LEU 105)

Oligo 49: 5'-AGTGACTATGAGGAC-3' (TYR 145)

Oligo 50: 5'-AGTGACTAGGAGGAC-3' (STOP 145)

Oligo 51: 5'-GACTGCGTCAATATC-3' (VAL 180)

Oligo 52: 5'-GACTGCATCAATATC-3' (ILE 180)

Oligo 53: 5'-TCGAGCATGGTCCTC-3' (MET 232)

Oligo 54: 5'-TCGAGCAGGGTCCTC-3' (ARG 232)

Oligo JC14: 5'-TGTGCACGACTGCGT-3' (ASP 178)

Oligo JC15: 5'-TGTGCACAACTGCGT-3' (ASN 178)

Coordinates within the *PRNP* gene of the other oligonucleotides used in this method were: Oligo 3, 85-111; Oligo 2, 840-816; Oligo 5, 100-124; Oligo 6, 448-427 (coordinates according to Kretzschmar *et* al.[176]).

The techniques were developed by Prof. John Collinge and Dr. Mark Palmer, and applied by Tracy Campbell and Katie Sidle.

During the last year of this study (from May, 1993 to end-April, 1994) following counselling of relatives by the author prior to obtaining blood for *PRNP* genome analysis, they were requested to indicate whether the result of the analysis should be made known to them. This was the recommendation of a MRC-sponsored meeting into the ethical issues surrounding *PRNP* analysis in cases of CJD (where the possibility of a mutation being found in the absence of a family history of neurodegeneration existed). The responses were noted for analysis.

Comparison of codon 129 status between definite/probable and other cases was performed by Chi-square. Clinical, electrophysiological and neuropathological characteristics of sporadic CJD cases encoding Met/Val at codon 129 of the *PRNP* ORF were considered, and compared with cases homozygous at this site using Chi-square. The same statistical technique was used to analyse the requests for results of genome analysis by relatives.

RESULTS

1.1 The study population

Between May, 1990 and April, 1994, 418 cases were referred to the U.K. national surveillance unit. The sources of referral have been described previously, and all suspect cases were British. Of these, a total of 241 cases were identified as being suitable for the current study. One hundred and seventy had definite or probable CJD, 41 cases were classified as other and 30 cases as other# (vide supra). The number of males:females in the definite and probable group was 73:97 (approximate ratio 1:1.3). The definite and probable group contained the following numbers of sporadic, familial and iatrogenic cases:

Sporadic	144
Familial	14
Iatrogenic	12.

The incidence of CJD in the U.K. over the period of study was therefore 0.773/million/year.

The ages of onset and disease durations of sporadic, familial and iatrogenic CJD are tabulated in Table 5. The number of patients for whom information is available in each group is indicated in parenthesis. Patients with familial disease presented about ten years earlier, and had disease durations approximately twice as long as patients with sporadic disease. As might have been anticipated, patients suffering from iatrogenic disease were younger, but their disease durations were not significantly longer than in sporadic cases. The clinical features of each of these groups are analysed separately.

2.1 Sporadic CJD

The distribution of the age of onset of sporadic CJD cases is shown in Figure 4. The distribution appears bimodal, with two peaks centred around the mid-sixties and mid-seventies. There is no increase in the number of cases with increasing age, and this remains true even after adjustment for the falling population with age (data not shown). The clinical, neuropathological and molecular biological characteristics of the patients in the two groups did not vary. There was a weak correlation between age of onset and disease duration, with younger patients tending to survive longer (r - 0.2822, p = 0.0008; Figure 5).

2.1.1 Clinical characteristics

The first clinical sign or signs, the early clinical features and the features noted during the course of the illness are tabulated in Table 6. The characterisation of cognitive impairment requires some clarification. Given the rapidly progressive nature of the illness, at the time patients were examined they were usually globally demented and the identification of specific neuropsychological deficits was impossible. The decision on which aspects of cognition were impaired at presentation and at different stages of illness was based retrospectively on the appraisal of case records. Using these recorded observations, the author decided which of four crude modalities of cognition had been affected (personality, behaviour, memory and orientation). In the analysis of these data, in addition to considering what proportion of patients had each specific aspect of impairment at different stages of disease, an overall estimate of what proportion had *some* aspect of cognitive dysfunction at each stage was also made. A similar retrospective and record-based approach was used to document which patients had had abnormalities of other "higher cerebral" functions, including dysphasia, dyspraxia and visual agnosia. When such abnormalities were identified in

the entire sporadic group (mean 7 months; median 4 (range 1 to 28) months). There were 9 patients with cerebellar incoordination but no apparent cognitive impairment at this stage. Theoretically patients with the Brownell-Oppenheimer variant (of progressive cerebellar incoordination, vide supra) should be included among these. Patients with such presentations are listed separately in the CJD surveillance unit, and cross-referencing revealed 3 cases included in both lists, 3 cases of CJD with progressive cerebellar incoordination not identified in this series (but all of whom had a "pure" cerebellar disorder at presentation), and 6 patients identified here who were not deemed to have suffered from this form of CJD (J. MacKenzie, personal communication). While emphasising the inevitably subjective nature of such classifications, these observations also indicate that this variant form of CJD is rare (probably not accounting for more than 4% of sporadic cases). Finally, bulbar dysfunction was identified in only 3 cases (2%) at this early stage.

As the disease progressed in this group of sporadic cases, multi-focal cerebral dysfunction was the norm, and further sub-division on the basis of the pattern of cerebral dysfunction was not feasible. Cognitive impairment was probably universal, being not recorded in this series in only 2 cases (present in 99%). The other clinical criteria used for classification (vide supra) were identified frequently:

Myoclonus	122 cases	85%
Cortical blindness	75 cases	52%
Akinetic mutism	107 cases	75%
Pyramidal signs (Py)	88 cases	62%
Extra-pyramidal (Par)	74 cases	52%
Cerebellar (C)	122 cases	85%
(Py, Par or C	139 cases	97%).

It is of note that primitive reflexes were noted in 83 cases (58%) and motor dyspraxia was present in 79 cases (55%). Paratonic rigidity in isolation was recorded in 55 cases (38%), but is likely to be an underestimate (due to its misclassification in the pyramidal or extrapyramidal categories). There were some differences in the frequency of these criteria between definite (pathologically proven) and probable (electrophysiologically proven) cases (Table 7), with myoclonus, cortical blindness and akinetic mutism all being recorded more frequently in the latter group. However, this difference did not reach statistical significance (Chi-squared = 5.256, df = 5, p = 0.3854). This observation is of some importance in relation to the validity of clinical criteria in epidemiological studies of CJD, and will be discussed later. Even in advanced disease, features such as bulbar dysfunction, seizures and respiratory depression remained unusual, with respective frequencies of 8%, 13% and 15%.

The disease durations of these cases are presented in figure 6. As noted previously, the mean and median disease durations are 7 and 4 months respectively. There is a suggestion that the distribution is bi-modal, with a tail of patients succumbing after 12 months. A further small number of patients are noted to have a disease duration in excess of 24 months. The relative proportions of patients with long disease durations are:

> 12 months	14/139	10%
> 24 months	6/139	4%.

Table 8 is a summary of the clinical, pathological and molecular biological data on these cases. In view of the previous observation of an inverse relationship between disease duration and age of onset in sporadic CJD, a younger mean age of onset might have been expected here. The mean age of the group was 62 years (range 44 to 77, median 60), and comparison of the ages of onset between short (<12 months duration) and long (≥12

months) duration cases revealed no significant difference by Mann-Whitney U (z - 1.7811, corrected for ties; p = 0.0749). During the course of illness, there was nothing remarkable about the clinical features of these cases. The first clinical features were studied in more detail, particularly as this might have enabled the identification of long duration cases at the time of their presentation. In fact the proportions of patients presenting with cognitive impairment alone, cerebellar features alone, a combination of cognitive and cerebellar disturbance, and cortical blindness were 40%, 30%, 10% and 5% respectively. It will be recalled that these proportions are almost identical to those noted for the sporadic group as a whole. Where it was performed, EEG was diagnostic (showing periodic sharp wave complexes) in 33% of cases. *PRNP* genome analysis was performed in 6 cases, and of these 5 were Met homozygous at codon 129 and one was heterozygous. Neuropathological examination always revealed diffuse spongiform change, and in 2 (out of 10 cases) anti-PrP positive amyloid plaques were noted. The significance of the EEG, molecular biological and pathological features of this group will become apparent later, but for the present there appears to be little that distinguishes this group of patients from the entire group of patients with sporadic CJD (apart from their longer duration of disease).

2.1.2 Investigations

EEG, liver function test, CSF, and CT/MRI data were gathered systematically from patients in the study. The results are summarised in Table 9. In the case of EEGs, strict inclusion criteria were used. Examples of recordings classified as typical are shown in Figure 7. The two recordings shown in Figure 8 are from the same patient (with pathologically confirmed CJD) and were performed less than a week apart. There is a clear progression in the appearances to become typical, emphasising the importance of repeating recordings. The recording shown in Figure 9 although suggestive was

classified as atypical. The danger of considering suggestive recordings as typical is illustrated by the examples shown in Figure 10. The patient was found to have ATD at autopsy. *The proportion of cases with pathologically confirmed CJD in which the EEG was typical was 35%.* Correlation between clinical features and EEG appearances was attempted. 48% of patients with <12 months disease duration and 33% of patients with ≥12 months disease duration had typical EEG appearances, but this observation was not statistically significant (Chi square = 0.781 (with Yates' correction), df = 1, p = 0.3769). When myoclonus was present, EEG was positive in 49% of cases; when myoclonus was absent, EEG was positive in 20% of cases (Table 10). The total number of cases without myoclonus was small, and the result did not reach statistical significance (Chi square = 3.457 (with Yates' correction), df = 1, p = 0.0630).

Abnormalities of liver "function" have been alluded to previously, and this systematic examination has confirmed this finding in almost half of all cases. Overt liver failure was not present in any of the cases, however, and the abnormalities usually amounted to a mild elevation of the hepatic enzymes. Serial studies were performed infrequently, but the impression gained was that the elevations were transient.[178,179] Figure 11 is an illustrative example of this phenomenon. At autopsy of this case, CJD was confirmed and the liver was found to have fatty change.

The abnormalities in the CSF were usually elevations in protein content. In this study, recordings above 0.4 grammes per litre were considered abnormal. Although a value of 0.92 was noted in a single case, the elevations were usually more modest. CSF examinations were rarely repeated, but in one case values of 0.34 and 0.76 were recorded approximately a month apart. At the second recording, however, 6225/mm^3 red blood cells were

present, making the interpretation difficult. A leucocyte response was *never* found.

The finding of abnormal CT or MRI findings in almost 60% of cases was surprising. Usually comments such as "mild atrophy" were present in reports. Whether these interpretations were influenced by the clinical data provided is unknown. Where scans were reviewed personally, a subjective impression of cerebral atrophy was sometimes formed, but this was not validated by comparison with age-matched controls. Patients with long duration disease might be expected to show more pronounced cerebral atrophy, and in order to investigate this the proportions of cases reported as abnormal in short and long duration cases were identified. The respective values were 56% and 67%, a finding which was once more statistically insignificant (Chi square = 0.340 (with Yates' correction), df = 1, p = 0.5598). MRI abnormalities have been reported in CJD. The extensive white matter degeneration reported by Uchino *et al*[180] may be specific for the Japanese panencephalopathic variant of CJD (vide supra). In reports from Western countries, high T2-signal lesions in the basal ganglia have been described.[181,182] In this study, three patients with sporadic disease had similar findings. Clearly, the abnormality is not universal in CJD, and it has been suggested that the finding may be specific for cases of CJD with pronounced extra-pyamidal features.[183] Of the three patients here, one presented with Parkinsonian features, but the other two had cognitive impairment alone. Of the latter, one patient did not exhibit any extra-pyramidal features during the course of her illness.

3.1 Familial CJD

In Table 11, clinical, electrophysiological and pathological data from cases of familial CJD identified in the study are presented. In addition to the earlier observations, on the younger age of disease onset and the longer disease duration in these cases, there are several points of interest. The estimate for familial CJD based on this study population is 8%. However, this is likely to be an under-estimate as not all cases of familial CJD will be referred to this unit, given its interest in sporadic disease. Furthermore, as referrals are based on the clinical suspicion of CJD, familial cases (which tend to have atypical clinical forms) will be referred less frequently. In fact, based on the total number of cases which have been screened for *PRNP* mutations, the best estimate for familial CJD is *16%* (13 out of 81 screened cases, excluding 5 screened cases of iatrogenic CJD). It should be noted that the patients bearing reference numbers 122 and 165 are from the same pedigree (first cousins).

The clinical diagnosis of CJD tended to be made in cases with the codon 200 (Glu - Lys) mutation and also in cases with extra base pair insertions in the octapeptide repeat region. Diagnostic EEGs were only obtained in cases with these types of mutations. However, the suspicion of CJD must have been sufficiently high for clinicians to have referred the cases in this study to the unit in the first place. This point is particularly salient in relation to the point mutations at codons 102 and 178. In both cases of FFI, bearing the codon 178 (Asp - Asn) mutation in association with Met at codon 129 of the mutated allele, the referring Neurologists suspected CJD. Although insomnia was a prodromal feature in both cases, dysautonomia was not marked, and the presence of cognitive impairment, dyspraxia, chorea, myoclonus, cerebellar incoordination and akinetic mutism (features present in both patients) would have been consistent with CJD. The neuropathological examinations in both

cases, however, were conclusive. In case 145, which was found to carry the codon 102 (Pro - Leu) mutation "diagnostic" of GSS, the clinical presentation was entirely in keeping with CJD. Even on neuropathological examination, the anti-PrP positive amyloid plaques (which were present, Figure 12a) fell short of the large, multi-focal aggregates described in the literature. The clinical phenotypes associated with this mutation appeared to vary from case 145 with an illness similar to CJD, to case 234 which resembled ATD (with cognitive impairment but *no evidence of cerebellar incoordination*). The mechanisms underlying this phenotypic variability are unknown. There was insufficient data on codon 129 genotype in these cases to investigate the effect of this site. There was a single case with a codon 117 (Ala - Val) mutation. In this patient the phenotype was consistent with GSS, and not the "telencephalic" variant (there being no pyramidal or extra-pyramidal signs).

The two cases bearing 144 base pair insertions in the octapeptide repeat region were not related. Furthermore, the *nucleotide sequence* was different in the two cases, and it seemed unlikely that they could have evolved from one another. Intriguingly, the amino acids encoded by the extra base pairs were identical in the two pedigrees, and this may have accounted for the similarity in the variety of clinical diagnoses attached to affected members of the families through the years.[89,90] The brother of case 173 was thought to have died from Huntington's disease thirteen years previously. Re-evaluation of his neuropathology confirmed CJD.[90]

The variable neuropathological findings in affected members of case 301's pedigree have been mentioned.[134,135] In his case, routine histochemistry revealed no abnormalities, but staining with anti-PrP antibody revealed punctate positivity (data courtesy of I. Janota, Institute of Psychiatry, The Maudsley Hospital, London). In this pedigree, the allele bearing the extra

base pair repeats always encoded Met at codon 129. Members of this extensive pedigree whose normal allele also encoded Met (homozygous at codon 129) had a younger age at death than members whose normal allele encoded Val at this site.[95]

The issue of family history is important, and as seen in Table 11 a positive history was obtained in a large proportion of cases (11 of the 13 cases for whom this information was available, or 85%). However, it is important to emphasise that the family history rarely suggested CJD *per se*. More frequently, vague references to disorders such as "Parkinson's disease", "multiple sclerosis" and "motor neurone disease" in other family members were made. In comparison, in the group of patients without *PRNP* mutations the proportion of cases with positive family histories of neurodegeneration was 26% (Chi square = 13.650 (with Yates' correction), df = 1, p = 0.0002).

Finally, in Table 12 the clinical characteristics of familial cases *as a group* have been summarised, at presentation, early in the illness and during the course of illness. In order to detect any differences in features between sporadic and familial cases, Spearman rank correlation of the relative frequencies of features at each stage has been performed. The data are included in Appendix 4: for each characteristic the proportions of sporadic, familial and, for a later analysis, iatrogenic cases manifesting this are tabulated. Columns 1 to 3 represent the frequencies at presentation, columns 4 to 6 the frequencies early in the course and columns 7 to 9 frequencies during the illness. According to the analysis, if there is a difference in the clinical phenotypes of the two forms of CJD at any stage of illness, a non-significant p value would be recorded. Given the manner in which the presence or absence of features was recorded for analysis, the relative magnitude of type 1 errors (including features as present when absent) is

likely to be greater than that of type 2 errors. The results of the analysis have been summarised in Table 13. It appears that there is no difference in the clinical characteristics between sporadic and familial cases referred to this unit, at any point of the illness.

4.1 Iatrogenic CJD

The causation of CJD in the twelve cases of this type in this study was as follows:

Cases 072, 025, 127, 160, 260, 274, 279, 308 and 255	HGH
Case 029	HGnH
Cases 054 and 333	Dura mater grafts.

Clinical data was available on all cases except case 255. The dura mater recipients presented at 44 and 46 years, and had disease durations of 5 and 11 months. Their clinical illnesses were similar to sporadic CJD. EEG was typical in case 333, but not in case 054.

The clinical characteristics of the CJD cases who had received treatment with HGH/HGnH, and for whom clinical data was available, is presented in Table 14. The striking feature is that almost all cases presented with cerebellar incoordination, and that early in the course this abnormality was invariably present. During the course of the illness, cognitive impairment was recorded to a variable extent, and 4 of the 9 patients (44%) reportedly never had features of this. Clearly, as these patients were not clinically monitored throughout their illness for the development of cognitive deficits, this observation needs to be interpreted with some caution. Nevertheless, 99% of sporadic cases were reported to have cognitive impairment (using similar

methods of ascertainment) during their illness. The implication may be that even when cognitive impairment develops in iatrogenic CJD via peripheral routes of inoculation, it is subtle and/or qualitatively different.

In Table 15 rank correlation of clinical characteristics at various stages of the disease has been performed between this group of patients and patients with sporadic disease. During the *early* part of the illness, a statistically detectable difference in clinical characteristics was present.

5.1 Non-CJD

A total of 41 cases with (eventually) pathologically confirmed non-CJD were referred to the unit during the period of study. With one exception, the diagnosis of CJD was entertained by the clinicians responsible for the patients during their terminal illnesses. The neuropathological diagnoses in these patients are presented in Table 16. The case of "spongiform myelinopathy" was in a girl of 15 years who collapsed and died a few hours later. Her case was referred to the unit by the OPCS, and further enquiries including examination of the post mortem report revealed this to be a case of sudden death associated with *white matter* disease which was unlikely to be confused with CJD clinically. This case and four others (with diagnoses of ATD (3) and ATD with multi-infarct disease (1), in whom clinical data was unavailable) were excluded from further analyses. As can be seen, the majority of patients without CJD had ATD either in isolation or in association with another neurodegenerative process (58%; 23/40).

The mean age of presentation for the group without CJD as a whole was 66 years (median 65; range 45-85). The mean disease duration in these patients

was 31 months (median 16; range 1-151). When compared with the sporadic CJD group, the disease duration in these cases was significantly longer (Mann Whitney U, z (corrected for ties) - 4.8675, $p < 0.0001$).

In Tables 17 to 19, the clinical characteristics at presentation, during early disease and during the course of illness in pathologically confirmed non-CJD cases (n = 36) have been compared with CJD cases with sporadic (n = 143) and familial (n = 14) disease. The CJD cases were considered together as information on *PRNP* genotype would not normally be available at the time the clinical diagnosis is made, and as noted the clinical characteristics of sporadic and familial CJD cases do not differ. As well as estimating the sensitivity and specificity of individual clinical characteristics, positive and negative predictive values have been calculated.[184] It has to be stressed that these calculations are based on a large number of CJD cases, but a very small number of non-CJD cases. The latter group was also non-homogeneous, and the relative importance attached to each individual clinical characteristic would have been heavily influenced by the frequency with which they occured in this "control" population. The positive predictive value in this context is probably meaningless, as the population prevalence of CJD is so low. In other words, even if a particular clinical characteristic had a high positive predictive value here, when applied to the whole population the number of false positives picked up on the basis of this sign would far outnumber the number of true CJD cases.

Nevertheless, there are several interesting points that emerge:

1. At presentation, the only clinical features that appear to have any sensitivity are cerebellar incoordination and the various aspects of cognitive impairment. However, all the features have high specificities

at this stage, with "disorientation" having the (comparatively) lowest value of 0.6944.

2. During early disease, incoordination remains the most sensitive marker of CJD, and its specificity remains high. All the aspects of cognitive dysfunction remain sensitive with fair degrees of specificity. Interestingly, motor dyspraxia emerges as a feature with moderate sensitivity, as do myoclonus and cortical blindness. However, the latter two have superior specificities.
3. During the course of illness, most of the diagnostic criteria emerge with high sensitivities. In addition to the various markers of cognitive dysfunction, myoclonus, cerebellar incoordination, akinetic mutism, pyramidal dysfunction and cortical blindness all have sensitivities greater than 0.5. Muscle wasting is an exception, with a sensitivity of 0.1656. At this stage, primitive reflexes and motor dyspraxia are also found to have high sensitivities. The specificities of the traditional diagnostic criteria were high with the exception of markers of cognitive dysfunction (due to the high prevalence of "dementia" in the non-CJD group) and, surprisingly, myoclonus. ATD with myoclonus is an important differential diagnosis in CJD,[47] and of the 19 cases with ATD in the non-CJD group for whom clinical data was available, this characteristic was present in 11. The specificities of primitive reflexes and motor dyspraxia were more modest, but paratonic rigidity emerged as a feature with an impressive specificity of 0.7222 (sensitivity = 0.3949).

As indicated previously, at the time a patient is suspected of having CJD and the clinical signs are elicited, the eventual outcome is unknown. The exact diagnosis in those patients who either recover or whose condition does not progress (designated other# in this study) is unknown. The group also

includes a small number of patients who died (without post mortem examinations), but the last available data on these subjects suggest that CJD is untenable. Not only is the group likely to be heterogeneous, but CJD cannot be ruled out for certain. It is because of the latter factor in particular that this group has been excluded from the analysis of clinical features between CJD and non-CJD cases. However, in order to validate the observations regarding clinical signs (at the time a case is initially assessed), a comparison between the relative frequencies of these signs at presentation, early in the course and during the illness has been made between other and other# cases.

At presentation, there were no differences between the two groups (Table 20). Early in the illness, pyramidal dysfunction, cortical blindness and incontinence were observed more frequently in the pathologically-confirmed non-CJD group (Table 21). During the course of illness, these features no longer remained significantly different, but facial weakness, akinetic mutism and (not surprisingly) respiratory depression were commoner in the "other" group (Table 22). These results imply that the conclusions drawn about the relative sensitivities and specificities of the various clinical signs above are valid when considering patients with suspect CJD during life.

6.1 Clinico-pathological correlation

The information was collated as described in the Methods section, and is included as Appendix 5. Data from 73 sporadic cases of CJD was available for analysis. The results from comparing clinical and pathological features in each area of brain in these cases are presented in Table 23. Overall, the basal ganglia were the most likely area of brain to show sponge (93% of

cases examined), followed by the cerebellum (92%), the temporal lobes (86%), the occipital lobe (81%),and the frontal and parietal regions (66% in both). The brainstem was the area of brain least likely to reveal spongiform change, this being present in 12% of cases only. In all areas of brain except the frontal lobes, there was no significant correlation between clinical features and sponge distribution. This poor correlation could be attributed in most regions to the lack of clinical features accompanying spongiform change. For example, of the 68 cases showing sponge in the basal ganglia, 38 had no clinical features of basal ganglia dysfunction. Approximately a third and a quarter respectively of cases with clinical signs of parietal and frontal lobe dysfunction failed to show sponge in these regions. It is important to stress that the assessment of pathology did not include other features such as neuronal loss or astrocytosis. The descriptive pathology reports did not enable quantification of the spongiform change either.

Eleven cases contained anti-PrP positive amyloid plaques. This approximated to *15%* of sporadic CJD cases in this unselected series. Clinical, pathological and molecular biological data on these cases are presented in Table 24. The mean disease duration in this group was 11 months, but would have been influenced extremely by the single case with a duration of 62 months. The median disease duration was in contrast 4 months, which was identical to the median disease duration of the sporadic group as a whole. Analysis of the presenting characteristics of these cases revealed that 55% had a purely cerebellar presentation and that 45% presented with some aspect of cognitive dysfunction alone. When compared with the presenting features of the rest of the sporadic group, this observation did not reach statistical significance (Chi squared = 5.030, df = 4, p = 0.2843). Characteristic EEG findings were present in only 2 of the 10 cases for whom this data was available, but comparison with the rest of the sporadic

group did not yield a statistically significant result (Chi squared, with Yates' correction = 1.896, df = 1, p = 0.1685). Of the four cases for whom molecular biological data was available, two were MV, one was VV and one was MM.

Finally, a single case in this sub-group had co-existing ATD. This was a 75 year-old female with an estimated disease duration of 5 months, but her family members had been aware of memory lapses and odd behaviours which were mild and not rapidly progressive for almost 18 months prior to the onset of her "acute" and terminal decline. The clinical course of her CJD was unremarkable, and her EEG was characteristic. She was Met homozygous at codon 129 of *PRNP* ORF. In contrast to previous reports,[185,168] this patient did not have a "long" duration of disease, and when her history was carefully reviewed there was a distinct change in her cognitive and neurological systems with the presumed onset of CJD. The coincidence of ATD with CJD is difficult to gauge based on this series, but the roughly 1% rate is compatible with chance concurrence of the two disorders.

7.1 Molecular biology

Data on familial cases (bearing mutations of the *PRNP* ORF) has already been presented. For the familial group as a whole, there was no significant difference in age of onset or disease duration when cases which were Met homozygous at codon 129 (n = 6) were compared with cases which were heterozygous at this site (n = 3) (Table 25).

Excluding the familial cases, *PRNP* ORF codon 129 genotype data was available in 89 cases:

Sporadic CJD	68

Iatrogenic CJD	5
Other (non-CJD)	16.

In sporadic CJD cases, 84% were Met homozygous; in non-CJD cases 50% were Met homozygous (Figure 13). The distribution of Met and Val at this site of common polymorphism is different between sporadic CJD and non-CJD cases (Chi squared = 8.527, df = 2, p = 0.0141). In an alternative analysis of some of these data, we found that *both* Met and Val homozygosity were significantly associated with the development of sporadic CJD (the relative risks of Met/Met : Val/Val : Met/Val being 11 : 4 : 1).[102] Here, this finding was not reproduced. In the published work, the polymorphic status of the definite and probable cases was compared with that of the normal Caucasian population. (The comparison of "possible" and "other" cases with the normal population revealed no significant difference.) It should also be noted that in the only other two population-based analyses of codon 129 status in sporadic CJD, Met homozygosity alone was implicated as a risk factor.[101,200] The explanation for this apparent discrepancy is unclear, but may be due to the infrequency of Val homozygous sporadic CJD cases and the relatively lower level of risk that this genotype confers compared with Met homozygosity.

The comparison of ages of disease onset and disease durations in sporadic CJD cases between Met/Met, Met/Val and Val/Val cases is presented in Table 26. There appears to be no difference in these parameters depending on genotype.

The clinical and pathological features of sporadic cases encoding Met/Val or Val/Val at position 129 are summarised in Table 27. In this group of patients, cerebellar dysfunction as a *presenting feature* was commoner when compared with the presenting features of patients who were Met homozygous

at this site (Figure 14; Chi squared = 15.689, df = 4, p = 0.0035). Furthermore, EEGs in these patients were rarely diagnostic (Chi squared, with Yates' correction = 10.254, df = 1, p = 0.0014). Neuropathological examination in these cases was more likely to reveal anti-PrP positive amyloid plaques (Figure 15; Chi squared, with Yates' correction = 4.078, df = 1, p = 0.0434). In summary, sporadic cases of CJD that are either heterozygous or Val homozygous with respect to the amino acids encoded at codon 129 of *PRNP* ORF, have identical ages of disease onset and durations of disease to Met homozygous cases. However, they tend to present more frequently with cerebellar dysfunction, rarely manifest typical EEG appearances and are characterised more frequently at neuropathological examination by anti-PrP positive plaques. It should be stressed that these amyloid plaques are quite different from the multicentric lesions "characteristic" of GSS. In these sporadic cases, the lesions are distinctive unicentric deposits (Figure 12b).[112,113]

In the five cases of iatrogenic CJD for whom codon 129 genotype data was available, two HGH recipients were Val homozygous, one HGH recipient was heterozygous, the HGnH recipient was Val homozygous and one of the dura mater graft recipients was Met homozygous at this site.

7.1.1 Counselling data

In Table 28, requests for the results from *PRNP* genome analysis by relatives have been noted. Overall, 73% of relatives expressed a wish to know the result. Requests did not appear to be influenced by the presence of a family history of neurodegeneration (p = 0.6273), whether the respondent was a first degree relative (p = 0.3069), or the family's level of education (p = 0.3354). (All calculations were performed by Chi square test, assuming Yates' correction and df = 1.)

DISCUSSION

1.1 The study design

In this study, an attempt has been made to analyse the clinical features of CJD systematically. Bias in case ascertainment has been minimised by including consecutive definite and probable cases of CJD referred to the UK national surveillance unit over a defined period (1990-1994). The period of study coincided with an unprecedented interest in the spongiform encephalopathies in this country.[186] Also, as indicated previously, cases are referred to the unit by a wide variety of sources, including neurologists, general physicians, geriatricians, psychiatrists and electrophysiologists. Neuropathologists and the Office of Population, Census and Statistics act as checks after death, so that there is virtually complete ascertainment of cases. The neurology and neuropathology communities in the UK are relatively small, and there are close links between members. There are regular contributions to the meetings of both groups from members of the surveillance unit. The work of the unit, therefore, has a high profile, and it is likely that most, if not all, identified cases of CJD are being referred. There is, however, a very real concern that cases of CJD with atypical presentations and courses are being missed. Clearly this must be occurring (if at all) in a systematic way in all countries where surveillance is taking place, as the epidemiological and clinical features of CJD in surveys worldwide are so consistent. The dependence on the Masters' criteria in particular may lead to the systematic under-ascertainment of cases of CJD not fulfilling these. There is some evidence in the present study that this is in fact unlikely to be the case. Firstly, cases designated "other" and "other#" in the study were suspected as having CJD at the time of referral; the "other" patients had an alternative neuropathological diagnosis established post mortem, and were clinically indistinguishable from "other#" patients (in whom the final diagnosis was unknown). It is likely that this entire group of patients, who do not fulfil criteria for

inclusion in the study as probable or possible CJD cases, *do not in fact have the disease*. If this group of patients who after all were referred by experienced neurologists as suspect CJD do not have it, it seems unlikely that CJD cases are masquerading with even more obscure presentations. Secondly, the identification of atypical variants of CJD such as FFI and (since the completion of the work presented here) vCJD by the unit argues strongly against the contention that the criteria for inclusion or exclusion in use are overly rigid.

It is conceivable that there are cases of CJD within the group of patients classified as "possible". These cases were not included in this study because their final status was uncertain (they could also have had a neurodegenerative process other than CJD). It is arguable that an unusual phenotype of CJD (not associated with periodic sharp wave complexes on EEG) has been missed by this approach. As will be argued later, the only reliable means of confirming or refuting this suggestion is by the systematic neuropathological examination at autopsy of all patients with neurodegeneration. However, it is unlikely that numerically these cases constitute a large group. The incidence of CJD during the period of this study based on the study criteria (0.773/million/year) is well within worldwide norms. Even if one assumes an annual incidence of 1.0/million/year, only a dozen cases have been omitted annually from the study, and not all of them would have had this putative phenotype. On balance, therefore, not only is it likely that virtually all suspected cases of CJD in the UK between 1990 and 1994 have been referred to the unit, but that the patients studied here constitute the bulk of the real cases.

The clinical data in the study has been collected by four experienced clinical neurologists, either by personal history-taking and examination, or by the careful

perusal of case sheets and investigations. The data was then entered into a standardised proforma. These measures have ensured a high degree of uniformity in the quality of the assimilated data. There is indirect support for this contention from the numbers of cases allocated to the various categories (definite, probable, possible and other) in the survey. Over the entire study period, the relative proportions in these categories have remained remarkably stable despite the involvement of different investigators (R. Will, personal communication). A similar observation was made during the current study: it was impossible to differentiate between the respective researchers on the basis of data available on individual patients.

Inevitably, there will be disagreement between observers on the presence or absence of (particularly subtle) clinical signs.[187] The estimation of this (kappa statistic) for the individual clinical features is beyond the scope of this study. Nevertheless, as considerable weight has been given here to the prevalence of cardinal clinical features, this deficiency has to be acknowleged. The neuropathological and molecular biological techniques have been performed and reported by highly experienced scientists. In comparison with the clinical data, the degree of uncertainty in these observations is low. The main caveat to this statement applies with respect to those cases which have been examined neuropathologically at other centres. However, it has been the policy of the unit to evaluate such cases independently, by the re-examination of wet tissue or by obtaining fresh sections from representative blocks. In all such cases, the report issued locally has been considered in preference.

The method for recording the presence or absence of clinical features in the current study may have over-estimated their prevalence. The use of a bi-nomial

variable would have encouraged the tendency to indicate as "present", when a feature was suspected or queried. In practice, this decision was rarely based on a single observation. For example, if a patient was suspected of being cortically blind, corroborative evidence of visual disturbance was sought from the history. Given that clinical data was gathered at one point in time (which may have been days, weeks or even months before death), and the availability of follow-up data was haphazard, the occurrence of some clinical features may have been underestimated. It is difficult to ascertain whether the presence of certain features was consistently underestimated by this mechanism: signs of bulbar dysfunction, for example, which probably occurs (if at all) late in the disease may have been ignored. In clinical practice too there is a tendency to be less clinically inquisitive, as it becomes clear that one is dealing with a progressive and untreatable condition, and the emphasis turns towards terminal care.

Data on investigation results was far from comprehensive. This is inevitable given the wide variety of sources from which patients were referred. Investigations such as EEGs were rarely repeated. The normal ranges for liver enzymes and CSF protein content were not always available. Given the design of the surveillance project, and the heavy reliance on the goodwill of referring clinicians, it is difficult to know how best to overcome these deficiencies. Given the nature of the disease, the ethics of repeating blood or CSF examinations for purely research purposes are dubious, especially when the patient is perceived to be in pain as a consequence. Despite these reservations, sufficient data on these investigations has been generated to enable the drawing of some broad conclusions.

In one part of the study, different stages of the disease process have been identified to aid classification. This is clearly a highly subjective exercise, there being no definite demarcations between "at presentation", "early course" and "during illness". Despite this arbitrariness, it has aided clinical speciation particularly at presentation. From this standpoint, it has been possible to make correlations with patients' neuropathological and molecular biological data. The present author had little difficulty in dividing the clinical information available in the proforma into these categories. It would be interesting to compare these numbers with those obtained by another clinician faced with the same data. If a degree of uniformity emerges, it will strengthen the clinical utility of this method of analysing the clinical presentation of CJD cases.

2.1 Sporadic CJD

2.1.1 Disease onset

The estimate for the median age of onset (based on review of the clinical history) in the sporadic CJD group was 65 years. This value is slightly greater, but consistent with recent large epidemiological surveys.[40,41] The apparent bi-modal distribution in the ages of onset (Figure 4), if a real observation, and not due to a chance phenomenon related to the relatively small number of cases studied, is worthy of investigation. It may, for example, indicate different mechanisms of disease acquisition in the population: the "younger" group developing the disease after a defined incubation period after exposure to the causative agent early in life, and the "older" group succumbing to an abiotrophic process with the passage of time. In the latter scenario, and indeed according to

the prion model, one would expect a progressive increase in the prevalence of sporadic CJD with increasing age.[96] This has not been noted in the present study. Inevitably, the accusation will be levelled that case ascertainment in the elderly is poor, but in unselective post-mortem studies there is little support for the suggestion that significant numbers of CJD cases in the elderly are being missed.[188,189,190] Furthermore, the design of the current surveillance study, its reliance on a wide variety of sources of referral and the unique clinical characteristics of the disorder all militate against this bias. Analysis of the clinical characteristics of elderly cases shows no difference from the group as a whole (data not shown).

The age of disease onset and indeed disease duration in the sporadic group were not associated with the common polymorphism at codon 129 (Table 26). It has to be noted however that the number of cases that were heterozygous or Val homozygous was small. The APOE genotype in this group of patients has not been studied. It is of note that the earlier French experience of the influence of the ε2 genotype on (longer) disease duration[142] has not been reproduced.[143] It is conceivable, as explained in the introduction, that the influence of this gene is limited to those cases of sporadic CJD exhibiting amyloid plaques.

2.1.2 Disease duration

There was an apparent correlation between age of disease onset and disease duration in sporadic CJD cases (Figure 5). This is not a startling or unexpected observation, and probably merely reflects the reduced capacity of the elderly to withstand the vicissitudes of a progressive dementing process. At a simplistic level, the prion hypothesis may be questioned on the basis of this observation. If the capacity for the development of CJD and its rate of progression are

governed by the degree of homology of interacting PrP molecules, should not a more rapid disease process ensue in those developing disease at a younger age?

As review of previous studies has shown, the apparent long duration of disease in young cases is partly attributable to the inclusion of familial cases in these series. This supposition is strongly supported in this study: familial cases presented on average 10 years younger (median = 55) and had a disease duration that was twice as long (median = 8 months, compared with a median disease duration = 4 months in the sporadic group). From the perspective of the prion theory, it may be argued that the degree of homology between PrP molecules available for interaction in familial disease is reduced, in that the *PRNP* ORF mutations are usually limited to a single allele. The presence of the mutation may still promote the conversion of PrP^{C} to PrP^{Sc} more readily, than that which occurs by chance in sporadic cases. In sporadic cases, however, once the conversion has taken place, the process can propagate more easily given the total homology between interacting species.

Fourteen per cent of the sporadic patients had disease durations greater than a year. Detailed analysis of the clinical, neuropathological and molecular biological features of these patients revealed nothing remarkable (apart from their disease durations). As indicated previously, and in contrast, some CJD cases have a rapidly progressive course, with an onset and evolution resembling stroke.[167] The observation that a single neurodegenerative process can exhibit such diverse characteristics is clinically fascinating, but it may simply be a reflection of the extremes of biological behaviour of this disorder in man.

2.1.3 Clinical characteristics

The method of study employed has enabled the classification of sporadic cases into phenotypic groups. The reliability of such techniques has already been questioned, but it is of note that the proportions of cases presenting in the current study with cognitive dysfunction in isolation, cerebellar dysfunction in isolation, combined cognitive and cerebellar dysfunction, and cortical blindness (40%, 30%, 10% and 10% respectively) are in keeping with the presenting features of cases in earlier worldwide surveys.[42,40,41] The roughly similar proportions of patients manifesting various clinical signs in Brown's 1994 study (in which all cases were experimentally transmitted)[41] and the current series is also notable (Tables 3 and 6). These observations lend considerable validity to the surveillance project in general and the current study in particular: the cases being studied constitute a group of patients with clinical features identical to those described in previous epidemiological studies and are representative of cases of transmissible human spongiform encephalopathy as conventionally understood.

The proportion of cases with Parkinsonian features (52%) is higher than that estimated by Will and Matthews (3%),[42] but in line with the estimates in Brown's series (56%,67%).[40,41] Will and Matthews classified rigidity in isolation as a pyramidal feature, whereas here increased tone throughout the range of movement of a joint (of "cogwheel" or "leadpipe" type) has been considered as a Parkinsonian feature. Parkinsonism is a difficult feature to establish in CJD as patients are not mobile and do not speak or write. Although it is an unusual *presenting* feature of CJD, as the pathological examinations attest, involvement of the extra-pyramidal system in CJD is not unusual. In contrast with the earlier studies, the presence of paratonic rigidity (gegenhalten) has been classified

separately, and recorded during the course of illness in 38%. Once again, it is unusual to detect this phenomenon early in the disease, but with disease progression (and presumed widespread cerebral degeneration) it emerges in a high proportion of cases. As indicated, it is likely that this phenomenon of increased tone has been misclassified as a pyramidal or extrapyramidal sign in a number of cases, making the current value an under-estimate.

The comparison with a pathologically-confirmed group of non-CJD cases has enabled closer scrutiny of the likelihood of the presence of various clinical features predicting CJD. In general, the Masters' criteria[38] emerged with high sensitivities, but perhaps this is unsurprising in that the same criteria were used in case selection. (This criticism can be levelled at all large epidemiological and clinical surveys of CJD worldwide.) "Muscle wasting" stands alone among the criteria in having poor sensitivity for the diagnosis of CJD, and lends support to the contention that on the whole cases previously described as "amyotrophic CJD" were patients with fronto-temporal dementia and motor neurone disease. Comparison of this pathologically confirmed group of non-CJD patients with a group of patients classified on the basis of the criteria as non-CJD but who have either improved or not succumbed to their neurodegeneration, revealed that the relative frequencies of diagnostic clinical features did not differ between these groups. The validity of the comparison lies in the fact that all the cases were referred to the unit as suspect cases of CJD. Therefore, the sensitivity and specificity of each sign can be used to gauge just how likely the presence or absence of this clinical feature makes the diagnosis of CJD likely, *in a patient being referred to a national centre with this diagnosis queried.*

The EEG data are a further point of interest. Again, its inclusion in the Masters' criteria will lead to an inevitable over-estimation of its diagnostic value. In the pathologically confirmed group, in whom this data was available, characteristic appearances (by this study's criteria) were noted in 35%. This is likely to be an underestimate of the total number of cases of CJD that have typical EEG findings, in that probable cases may not have come for autopsy as readily as those cases of suspect CJD in whom the diagnosis could not be established during life. In contrast to Will and Matthews' data,[42] there was no significant tendency for EEGs to be uncharacteristic in long duration disease. There was a suggestion that the EEG was likely to be negative in the absence of myoclonus (Table 10), but the result was not statistically significant. It is of note that the frequency of some clinical signs (cortical blindness, akinetic mutism, Parkinsonian features and myoclonus) was higher in the electrophysiologically confirmed group, than in the definite cases. The result did not reach statistical significance, but does suggest that a proportion of cases may not develop characteristic signs or EEG appearances. The over-reliance on the Masters' criteria (and especially electroencephalography) may consequently lead to the under-estimation of CJD cases. This observation has serious implications for the surveillance of CJD. If humans were to become susceptible to the agent causing spongiform encephalopathy via novel modes of inoculation, unusual and atypical disease phenotypes might arise. These in turn might be expected to manifest atypical EEG appearances.[161] The recent experience with vCJD is perhaps an illustrative example.[162] An over-rigid adherence to the Masters' criteria would lead to these cases being ignored. Perhaps the only certain means of avoiding this would be to perform neuropathological examinations of the central nervous system following autopsy in *all* cases of undiagnosed neurodegeneration during life. A policy such as this will have important implications on clinicians' and

neuropathologists' time, and neuropathological resources. This approach will have the useful spin-off of generating an enormous amount of clinical and neuropathological data on neurodegenerative processes in general.

At present, nevertheless, EEG remains the most useful diagnostic test in CJD. Contrary to Nevin *et al*'s early observations,[20] this test has a *high* specificity (it being rarely "positive" in an appropriate setting in conditions other than CJD).

Certain clinical features and phenotypes were confirmed as being rare in CJD. No cases of panencephalopathic CJD were identified. The comparative differences in the reported occurrence of this form of CJD between Japanese and Western series is unexplained. The Brownell-Oppenheimer variant with progressive cerebellar ataxia was also unusual, not accounting for more than 4% of sporadic cases. The relatively low prevalence of bulbar dysfunction and respiratory depression even in the advanced stages of CJD has been indicated, and possible explanations suggested. It is unlikely that a similar problem with ascertainment would apply to seizures (especially when generalised), and the reported (low) frequency of 13% is likely to be accurate. A proportion of cases may have received treatment with agents such as Clonazepam or Valproate for myoclonus, which may have suppressed seizure activity. It is worth bearing in mind that PrP null mice have demonstrated pro-epileptogenic electrophysiological abnormalities, implying a gain of function rôle for this protein.[146] In disease states, such as CJD, it is argued that such abnormalities will re-emerge. It is, therefore, slightly surprising that seizures are not more commonly encountered in CJD.

3.1 Differential diagnosis

The main differential diagnosis in the non-CJD group was ATD, accounting for 58% of cases in this group. In those cases of pathologically confirmed ATD for whom clinical data was available, a high proportion (58%) had myoclonus. The variety of other conditions in the non-CJD group was huge, and appeared to encompass virtually the entire spectrum of adult neurodegenerative processes (Table 16). The group as a whole had a disease duration roughly four times longer than in patients with CJD (median = 16 months), and this was perhaps the most useful discriminator. A single patient in this series had co-incident CJD and ATD. This association has been described previously, and probably represents a chance occurrence. Data gathered retrospectively from the patient's record could be used to identify the onset of rapidly progressive neurodegeneration (presumably CJD) on a background of subtle and slow cognitive decline.

4.1 Familial CJD

Although the estimate for the proportion of cases of CJD that was inherited based on large scale epidemiological studies was around 6%,[44,36] a true figure could only be arrived at by the systematic screening for all *PRNP* ORF mutations in an unselected, large population of CJD patients. This was achieved in the current study, and the estimate of 16% is in agreement with the experience in France.[101] The relatively younger age of onset and the longer disease duration in these cases have been alluded to.

A significantly greater proportion of familial cases (85%) was found to have a family history of neurodegeneration. Although it could be argued that knowledge of the *PRNP* genome analysis may have influenced the interpretation of the family history, it should be recalled that this information was on the whole collected prospectively (and before the molecular biological data became available). Furthermore, the family history recorded rarely implicated CJD by name, and more often referred to commoner neurodegenerative processes such as Parkinson's disease. The estimate for family history of neurodegeneration in cases without *PRNP* mutations (26%) was based on records of similar diagnoses in first degree family members. This observation has important implications for the assessment of family risk in individuals with CJD. The presence of a positive family history of neurodegeneration clearly increases the likelihood of an inherited form of CJD (relative risk = 3.24; 95% confidence interval = 2.02 - 5.17).

A wide spectrum of disease-associated mutations was recognised in the study (Table 11). One of the pedigrees was found to have a novel 144 base pair insertion in the octapeptide repeat region, and has been reported.[90] As mentioned, although the unusual disease phenotype of FFI has been stressed in the literature, both cases identified here were suspected of having CJD by their referring clinicians. The mutations described in association with GSS in the literature were found to have highly variable clinical phenotypes. At one end of the spectrum cases had clinical courses not dissimilar to CJD, while at the other cases resembled ATD. There was insufficient data on codon 129 genotype to draw any conclusions about the influence of this polymorphism on disease phenotype.

The case associated with the codon 117 mutation had a GSS-like disease phenotype. A recent case report of an individual with the same mutation is of interest: the clinical and pathological phenotypes of this case were also those of GSS and not the "telencephalic" variant.[191] In the recently reported case, as in all reported cases of codon 117-associated CJD, Val was encoded by codon 129 of the disease-bearing allele. In contrast to the mutation at codon 178,[106] phenotypic variability in association with this mutation does not appear to be determined by the polymorphism at codon 129. The polymorphic status of the normal allele could be responsible, but this seems unlikely on the basis of the relative homogeneity of clinical features in affected subjects from large pedigrees. The other possibility is that there are further sites of genetic influence on pathogenesis, as suggested in mink[192] and mice.[193] Clearly, further studies on the pedigree of case 309 are warranted in order to examine these issues.

In contrast with the work of Poulter and colleagues,[95] it was not possible to show any effect on age of onset or disease duration of the pooled familial CJD cases of the codon 129 polymorphism (Table 25). The explanation for this discrepancy is not clear, but may be due to the effect of this site either being limited to certain mutations or being identifiable *within* pedigrees only. It should also be noted that the data available in the current study is limited to only nine subjects. If the results of larger series confirm the lack of influence of this site on disease characteristics, this would not necessarily bring into question the concept of interacting PrP molecules as a key pathogenic mechanism. PrP molecules derived from mutated alleles may have more crucial structural characteristics which facilitate homologous interactions.

The pooled data on clinical features of familial cases failed to show any differences at presentation, early in the course or during the illness when compared with the sporadic group. This analysis does of course ignore the fact that in individual cases the suspicion of an inherited form of CJD may be raised by certain clinical features, such as insomnia in FFI. Nevertheless, it appears that it is untenable to base a diagnosis of inherited CJD on clinical features alone and without *PRNP* genome analysis. It was equally difficult to predict the type of *PRNP* ORF mutation based on the clinical phenotype. The typical CJD-like illnesses identified in association with the codon 200 mutation and in those cases carrying extra base pair insertions in the octapeptide repeat region were exceptions. The second case of FFI was not recognised clinically but the pathological findings were diagnostic (prior to the availability of the molecular biological data).

Recent studies on familial cases of progressive subcortical gliosis have revealed anti-PrP positive reactions, and linkage with chromosome 17 markers.[194] Examination of the *PRNP* ORF revealed *no* mutations. The place of this entity (which can also present sporadically) in the spectrum of human transmissible spongiform encephalopathies is uncertain, but if transmissibility is achieved and PrP is demonstrable on Western immunoblotting[195] there will be a pressing need to study chromosome 17 in greater detail to find sites of influence on *PRNP* or PrP processing.

5.1 Iatrogenic CJD

The clinical characteristics of nine cases of CJD acquired following inoculation of the agent outside the central nervous system were available for study. In keeping with experience elsewhere, these patients presented with or manifested early on features of cerebellar incoordination. Cognitive impairment was not recorded in just over half of the patients. The validity of the clinical staging is supported by the demonstration of a statistically significant difference in characteristics between these cases and sporadic cases in the early part of illness by Spearman rank correlation. (The difference in characteristics at presentation also approached significance.)

The median disease duration of the iatrogenic cases was similar to that of the sporadic group. From the point of view of the prion theory this is not a surprising observation: the recipient's PrP molecules will have no difficulty interacting with the PrP molecules in the inoculum (of human pituitary extract). The extent to which homology at codon 129 (between recipient and inoculum) needs to be satisfied for this interaction is unknown. Although the numbers were too small for statistical analysis, out of the five iatrogenic cases for whom molecular biological data was available four were homozygous at codon 129. Interestingly, all three cases with agent inoculation outside the central nervous system and who were homozygous (the fourth was heterozygous) encoded Val at codon 129. As discussed in the introduction, pooled data has confirmed the increased prevalence of Val homozygosity in patients who have developed iatrogenic CJD after receiving human pituitary-derived hormones.[100] Whether this is revealing something fundamental about prion biology or represents a chance phenomenon is currently unknown. One attractive hypothesis is that "matching" (with respect to the amino acids encoded at codon 129) between recipient and inoculated PrP

molecules may have led to the currently recognised cases. The human pituitary-derived HGH and HGnH were, however, obtained from pooled supplies and the molecular structure of the infectious particle(s) will never be known.

The observation regarding the short disease duration in iatrogenic cases is also relevant in terms of our appreciation of their clinical characteristics. Although recent descriptions of "peripherally inoculated" iatrogenic CJD cases have referred to a "kuru-like" illness, closer scrutiny of descriptions of kuru and analysis of the clinical characteristics of patients in series such as this reveal important distinctions. The disease duration of kuru in Hornabrook's experience was 12 - 18 months.[29] Also, myoclonus is not described in kuru patients, but was present in 7/9 "peripherally inoculated" cases studied here. The prominence of cerebellar features, the early lack of insight and the late development of dementia are features in common, and may (as is commonly suggested) imply a dependence of the clinical features on the route of inoculation. Nevertheless, the likening of illness in these cases to kuru is simplistic and probably inaccurate.

6.1 A comprehensive classification, and new diagnostic techniques?

6.1.1 Classification

Based on the foregoing, the human transmissible spongiform encephalopathies can be summarised as follows:

1. **Sporadic CJD**: "Classical" - presenting with cognitive dysfunction alone (40%)
presenting with cerebellar dysfunction alone (30%)
presenting with cognitive and cerebellar dysfunction (10%)
other presenting feature (10%)

Heidenhain variant - presenting with occipital blindness (10%)

Brownell-Oppenheimer variant - progressive cerebellar syndrome (rare)

Panencephalopathic - (rare)

2. **Familial CJD**: Phenotypes linked with 22 separate *PRNP* open reading frame mutations

Including Phenotype resembling sporadic CJD
Gerstmann-Sträussler syndrome
"Telencephalic" CJD
Fatal Familial Insomnia
Familial CJD with spastic paraparesis
Phenotype resembling Alzheimer's disease

But, considerable overlap and phenotypes not specific to mutations

3. **Iatrogenic CJD**: Central inoculation - resembles sporadic CJD

Peripheral inoculation - progressive cerebellar syndrome

4. **Kuru**

The classification is a clinical one, and may require revision on the basis of future developments in neuropathology and/or molecular biology. The nosology of entities such as vCJD, progressive subcortical gliosis and Alpers' disease (vide infra) is not established, but arguments can be put forward for the inclusion of each of them as a human spongiform encephalopathy. (*Transmissibility* has not been unequivocally established for any of these conditions, to date.) In time, therefore, the classification may require expansion.

6.1.2 Novel "diagnostic" techniques

The absence of a true diagnostic test in CJD has been pointed out. The results of investigations performed at the referring centres on the cases included for study here (Table 9) were compatible with results reported elsewhere. The results from the examination of CSF for the 14-3-3 brain protein of cases is not reported in this study, but a systematic survey of cases versus non-cases referred to the UK surveillance unit revealed that the specificity of this technique was low.[164] The German surveillance group has found the examination of CSF for not only this protein, but also the estimation of CSF neurone-specific enolase to be of diagnostic value.[196,197] The latter marker is known to be elevated in a variety of neurodegenerative processes affecting the central nervous system. As in the case of minor elevations in CSF protein (37% of cases had a CSF protein of greater than 0.4 grammes per litre in the current study), it may simply reflect neuronal cell death and therefore lack specificity.

The interest in MR abnormalities in this condition has been mentioned. In addition to the symmetrical pattern of high T2 signal arising from the basal ganglia, a recent paper has described a single case with an asymmmetrical pattern.[198] This case, interestingly, manifested corresponding (asymmetrical) abnormalities on electroencephalography and single photon emission computerised tomography. In the present study, even in those cases where MR examinations were performed, the finding was not universal. A symmetrical abnormality was noted in three sporadic cases, but no consistent features in their clinical characteristics were identified. Subsequent work from the unit has established the poor sensitivity of this imaging modality in making a diagnosis of CJD.[199]

The study confirmed Met homozygosity at codon 129 as a "risk factor" for the development of sporadic CJD (Figure 13), in keeping with Palmer *et al*'s original observation[99] and subsequent large series.[101,102,200] When Met homozygosity at this site is considered as a diagnostic test of sporadic CJD, and compared with the genotype at codon 129 of non-CJD cases, the sensitivity is 0.8382 and the specificity is 0.5000. To these apparently impressive figures must be added the cases of familial CJD that would be diagnosed by a comprehensive genotypic evaluation of the *PRNP* ORF. However, the positive predictive value of Met homozygosity at codon 129 in the diagnosis of sporadic CJD in the community is likely to be very low, given the rarity of this condition.

Finally, reference has been made in the introduction to a promising technique in which biopsy of tonsil tissue (at autopsy) showed positive anti-PrP staining and Western blot of tissue homogenate revealed protease-resistant PrP with physiochemical characteristics similar to protease-resistant PrP from previously diagnosed vCJD cases.[166] Not only does this require replication ante mortem in future vCJD cases, but it will be important to establish how often protease-resistant PrP is identified in the tonsils of cases of sporadic, familial, and iatrogenic CJD with central and peripheral routes of inoculation.

6.1.3 Ethical considerations

One other concern regarding the systematic surveillance of *PRNP* genotype in suspected cases of CJD pertains to the ethical dilemmas posed, particularly by the inadvertent discovery of a familial case. As noted, although these cases may present at a younger age and have a longer duration of disease, their clinical features are not different to sporadic cases. Furthermore, although a family history is present in a large proportion, it is rarely identified as CJD or accepted

as an autosomal dominantly inherited condition in which half of all offspring are likely to develop disease. *PRNP* genome analysis has already been undertaken in the screening of undiagnosed familial ataxic/dementing cases,[201] and for pre-symptomatic prediction in members of pedigrees with known inherited CJD.[202] A single case of pre-natal exclusion of GSS has also been reported.[203] In the current study, the acceptance rate for *PRNP* genome analysis was surprisingly high at 73%. Counselling was carried out by the author, usually after relatives had been informed about the progressive and incurable nature of the disease. At the interview, an information sheet summarising the main points covered was issued (Appendix 6), and the relative's signed consent was obtained. Relatives were firstly requested to give their permission for genome testing, and if this was given (as in all the cases) they were asked to indicate whether the result should be disclosed to them or not (Appendix 7). As noted, the decision to know the result of genome analysis was uninfluenced by the presence of a family history of neurodegeneration, the direct implications for the respondent or the family's level of education. In most cases, it was felt that the decision to know the result of genome studies was taken *with the belief that the test would show no risk to remaining relatives.*

7.1 Clinico-pathological correlation

The comparison of clinical features with the distribution of sponge in the appropriate brain regions in cases showed little correlation (Table 23). The methodological deficiencies inherent in this analysis which may have contributed to this observation have already been mentioned. If valid, however, the comparison raises several interesting issues. The distribution of sponge may not

reflect neuronal loss, which may be a more important determiner of cerebral dysfunction. The extent of involvement of the basal ganglia (93% of cases examined revealed spongiform change in this region) is notable. Although extra-pyramidal features do occur in sporadic CJD, they are not common: Parkinsonism, for example, was identified in only around a third of cases in this series (Table 6). This lack of correlation between pathology on the one hand and clinical disease on the other is matched by the lack of correlation between basal ganglia high T2 signal on MR imaging and clinical features in CJD cases. Of the three cases with this abnormality that were identified in the current study, one patient did not manifest any features of extra-pyramidal dysfunction. It is tempting to suggest even on the present limited data, that the abnormalities noted on MR are a non-specific reflection of spongiosis.

At a more basic level, the lack of correlation may be attributable to our incomplete understanding of function localisation within the cortex. Also, the degree of neuronal plasticity (which may vary for different cell populations) could have made the simplistic assignment of functions to brain regions meaningless. In view of all these reservations, the apparent correlation between frontal lobe dysfunction and sponge distribution (and a similar correlation in the cerebellum which approaches statistical significance) could have arisen by chance. Whether the association between neurone loss and sponge is different in these particular regions is unknown. There may also be fundamental differences between the behaviour of neurone populations in these regions (and their capacity to withstand disease processes), and the behaviour of neurone populations in regions of the brain where no correlation was found. The lack of correlation in the occipital cortex is particularly interesting, given the relative confidence with which blindness can be diagnosed and the early observations on Heidenhain's

syndrome (the tendency for there to be prominent vacuolation affecting the occipital cortex in these cases).[21,5]

7.1.1 Cases with anti-PrP positive plaques

In the cases studied, around 15% of sporadic CJD brains were found to exhibit amyloid plaques, staining positively with anti-PrP. The systematic examination of all cases in Edinburgh with immunocytochemistry is likely to make this observation accurate. Analysis of the clinical characteristics of these cases revealed that 55% *presented* with pure cerebellar dysfunction (an observation that failed to reach statistical significance when compared with the presenting features of the rest of the sporadic group). Typical EEG findings were recorded in only 2/10 cases, but again this observation was not statistically significant in comparison with the rest of the sporadic group.

There did appear to be an association between the presence of alleles encoding Val at position 129 and the presence of anti-PrP positive amyloid plaques (Figure15). Independent analysis of the clinical features in these cases revealed that they were more likely to present with pure cerebellar dysfunction (Table 27 and Figure 14) and that typical EEGs were less likely to be recorded (Table 27). The ages of onset and disease durations in these patients were no different from patients who were Met homozygous (Table 26).

This apparently distinct clinical and neuropathological phenotype of sporadic CJD raises an important issue. Does the phenotype result from the "susceptibility" of individuals carrying the triplet encoding Val at codon 129 of the *PRNP* ORF to develop CJD following contact with an (exogenous?) agent which has a tendency to produce amyloid aggregates? Alternatively (and in the present

author's view more plausibly), does it represent a marker of the disease process in individuals with this genotype? If the latter interpretation is correct, further study of these cases may reveal differences (from Met homozygous patients) in their handling of the disease agent, or in the response of their neuronal cytoarchitecture to the pathological process. It is of note that the rare Japanese cases with this genotype show similar unicentric anti-PrP positive aggregates, and that their cases are described as having "GSS" with progressive ataxia and atypical EEGs.[112]

Since the completion of this study, Parchi and colleagues have published a paper on sporadic CJD in which phenotypic variability was analysed according to the Met/Val polymorphism at codon 129 of the *PRNP* ORF, and the characteristics of PrP according to Western blotting and immunohistochemistry.[204] The total number of patients studied was only 19. They define four phenotypes on the basis of Met homozygosity, heterozygosity and Val homozygosity, and two isoforms of protease-resistant PrP. Interestingly, they too found that that the presence of codon 129 Val alleles was associated with ataxic presentations, that only 1/6 patients with this genotype had typical EEG appearances, and that all cases had "plaquelike and punctate" patterns of anti-PrP immunostaining. The authors conclude that their data argues in favour of distinct prion variants (probably defined by the two isoforms of protease-resistant PrP) affecting humans, although the rest of the phenotypic variability is (presumably) determined by the host genotype. In favour of these authors' hypothesis was the observation that the two types of protease-resistant PrP in Met homozygous patients produced two distinct phenotypes, with the "type 2" being associated with a longer duration of disease. However, this observation was based only on two cases.

8.1 Whither the prion theory?

It would be inappropriate to argue for or against the prion hypothesis purely on the basis of an observational study of the clinical characteristics of CJD. As it stands, however, the theory has some difficulties in accounting for some of the observations that have been made in this study, and elsewhere.

1. If younger sporadic cases are likely to have a more efficient polymerisation process involving homologous PrP molecules to account for their younger age of onset, why is the disease duration in these cases also not shorter? In fact these cases are noted to have a *longer* disease duration. Presumably, other factors such as the well being of organ systems, more efficient metabolism and better immune function all contribute to the enhanced survival, but it is notable that the neurodegenerative process (particularly if determined by PrP interactions) does not appear to have the dominant rôle.

2. If sporadic disease occurs purely as a result of the spontaneous conversion of PrP^{c} to PrP^{Sc}, why is there no progressive increase in age-specific incidence with increasing age? (The opportunity for this kind of transformation would increase with increasing age.) It has been argued that case ascertainment in the elderly is imprecise, and that cases are systematically missed even with intensive surveillance. An increase in elderly cases has been recorded during the period of the current UK surveillance study,[161,205] but the fall-off in age-specific incidence with increasing age continues to be noted. The latter observation has been confirmed in a recent study from Austria in which the latest available incidence value (for 1995) for necropsy-confirmed CJD was 1.25/million, implying near complete case ascertainment.[190] Over the whole period of study (from 1969 to 1995), the number of cases fell progressively from 19 in the group aged 60 to 64 years, to 2 in the group aged 80 to 84 years.

Furthermore, as already explained, in unselected post-mortem studies of the very old, the diagnosis of (unsuspected) CJD is rare.[188] It is likely therefore that the reduction in age-specific CJD incidence with increasing age is a real observation, needing modification of the prion protein theory to account for it satisfactorily.

3. If the homology of PrP molecules is so critically influenced by the amino acids encoded by codon 129 of the *PRNP* ORF,[95] why is there no observed tendency for sporadic CJD cases which are heterozygous at this site to have older ages of onset and/or longer disease durations (Table 26)? A similar lack of effect of this site of common polymorphism on age of onset and disease duration was observed in the pooled familial data. Once again, there may be other factors governing PrP interactions that have a more fundamental influence on age of presentation and disease duration, than the poymorphic status at codon 129.

4. If homologous PrP interactions are so crucial, would the population incidence of sporadic CJD not be determined by the prevalence of Met-encoding alleles (at codon 129)? In populations where Met homozygosity is virtually universal (such as the Japanese) the incidence of CJD is no different.[35]

5. Attention has been drawn to the difficulties experienced in attributing phenotypic variability in familial CJD cases to sites of genetic influence purely within the *PRNP* ORF.[107,194] If there are other sites of importance within the human genome that are implicated in familial CJD, PrP interactions may emerge as less critical in the susceptibility to the development and propagation of CJD in general.

These criticisms can be countered by the wealth of observational and experimental data which is in favour of the prion hypothesis. The recent modification of the theory in which another protein (protein X), which functions

as a species-specific molecular chaperone in the formation of PrP^{Sc}, is evoked may account for some of the discrepancies.[97] Could, for example, the putative protein X be encoded by a gene outwith *PRNP* in humans?

9.1 Can the BSE risk to humans be assessed clinically?

Following the period of study in this thesis, an apparently novel form of Creutzfeldt-Jakob disease has been identified. Fourteen cases of this vCJD have been confirmed to-date in the UK,[206] and a further case has been reported from France. As discussed in the introduction (page 32), vCJD appears to be characterised by unique neuropathological features and unusual clinical characteristics. In contrast with the data presented here on patients with CJD identified between 1990 and 1994, vCJD patients do appear to be quite different. However, it would be unwise to associate this phenotype as being aetiologically related to BSE purely on this evidence. (It is not: compelling laboratory data has emerged within the past year to support an association. Transmission of BSE by intracerebral inoculation into macaques produced strikingly similar pathological appearances,[207] and experimental studies on disease-related PrP isoforms from patients with vCJD appear to show physiochemical similarities between protease-resistant PrP derived from these patients and protease-resistant PrP from BSE tissue.[165,208]) It is likely that our appreciation of the spectrum of disorders associated with spongiform encephalopathy and their clinical presentations is incomplete. Alpers' disease is a case in point. This rare childhood progressive encephalopathy is associated with cerebral spongiform change,[209] and in certain hands has been successfully transmitted to laboratory animals.[210] Nevertheless, it is still not established whether this disease should be

considered as a human spongiform encephalopathy. Consequently, it is conceivable that vCJD is not a new phenomenon, and that these cases were not considered as a spongiform encephalopathy on account of their atypical presentations. Their identification at this point in time could have been secondary to the intensity of the surveillance effort in the UK. This seems an unlikely explanation, however, and intensive retrospective studies on post mortem tissue and on-going surveillance efforts in Europe and the US have not identified any other cases of vCJD (up to now). The geographical isolation of vCJD to the UK (mostly) need not imply an association with BSE either. Panencepahlopathic CJD as has been noted appears to be found in Japan only. Whether or not vCJD proves to be associated with BSE, if successfully transmitted, it will extend the spectrum of human spongiform encephalopathy as currently understood.

One key observation with respect to vCJD is the currently identified (young) age of onset. It is not readily apparent why BSE (if the aetiological factor) should be a particular risk to young humans. One concern is that these cases have come to the attention of clinicians simply because of their unusual age, and that cases presenting at older ages will be "diluted" by classical CJD cases. Formal descriptions of the clinical characteristics of vCJD are in preparation (M. Zeidler, personal communication), but presentations of CJD cases with pure psychiatric features or sensory symptoms should alert clinicians to the possibility of vCJD. In response to the question posed at the start of this section, clinical surveillance may not constitute the entirety or even the mainstay of the scientific effort for answering this important question. However, without continued clinical vigilance (backed up by the systematic neuropathological examination of central nervous system tissue) it is unlikely to succeed.

TABLES

Legend for Table 1: The transmissible spongiform encephalopathies

*Includes Gerstmann-Sträussler syndrome and Fatal Familial Insomnia

**These disorders are suspected or proven to be related to Bovine Songiform Encephalopathy

Table 1: The Spongiform Encephalopathies

Disorder	Species
Creutzfeldt-Jakob disease	Human
Inherited Creutzfeldt-Jakob disease*	Human
Iatrogenic Creutzfeldt-Jakob disease	Human
Kuru	Human
New variant Creutzfeldt-Jakob disease**	Human
Scrapie	Sheep/Goat/Moufflon
Transmissible mink encephalopathy	Mink
Chronic wasting disease	Deer/Elk
Bovine Spongiform Encephalopathy	Cattle
Feline Spongiform Encephalopathy**	Cat/Cheetah/Puma/Ocelot
Spongiform Encephalopathy of Captive Exotic Ungulates**	Kudu/Nyala/Oryx/Gemsbok/Eland

Table 2: Cases described by A. Jakob[14]

Patient	Age	Duration of illness	Clinical course	"Differential"*
1	51 years	12 months	Leg weakness Depression Ataxia Dysphagia and aphonia "Positive Babinski" "Pseudospasms", startle response	?Syphilis
2	34 years	1.5 months**	Psychomotor disturbance Incontinence Broad-based + spastic gait Limb rigidity Mask-like facies, slow + monotonous speech Epilepsy	
3	42 years	9 months	Disturbed ocular movements Disturbed speech + writing Limb ataxia + dysarthria Spasticity Visual hallucinations Muscle wasting	
4	43 years	~7 months	Leg weakness "Nocturnal delirium" Lower limb spasticity Urinary retention Psychomotor disturbance	?Malnutrition (NB ex-alcohol abuse)

* Differential diagnoses suggested by W.R. Kirschbaum.[17]

** Kirschbaum estimates the duration of disease in this patient to be 6 months.[17] Jakob's original case description states that the patient died 6 weeks after the onset of symptoms.[14]

Legend for Table 3: Comparison of clinical features at the onset and during the course of illness in three series

Table 3: Comparison of clinical features

Clinical feature	Will and Matthews, 1984[42]		Brown *et al*, 1986[40]		Brown *et al*, 1994[41]	
	Onset	Course	Onset	Course	Onset	Course
Cognitive impairment	21%	100%	64%	100%	69%	100%
Cerebellar dysfunction	19%	42%	34%	61%	33%	71%
Visual failure	9%	13%	17%	42%	19%	42%
Pyramidal disease	-	79%[a]	2%	43%	2%	62%
Extra-pyramidal disease	-	3%[a]	2%	67%	0.5%	56%
Lower motor neurone signs	-	3%	0.4%	11%	0.5%	12%
Seizures	?1%[b]	9%	0.4%	8%	0%	19%
Myoclonus	?5%[c]	82%	0%	88%	1%	78%

[a]Presence of rigidity alone was classified as "pyramidal"
[b]"Blackout attacks"
[c]"Involuntary movements"

Table 4: Cases of inherited CJD and their linked PRNP mutations

ORF codon	Amino acid change	Suggested phenotype	Reference
102	Pro - Leu	GSS	Hsiao, 1989[25]
105	Pro - Leu	"GSS" + spastic paraparesis	Kitamoto, 1993[74]
117	Ala - Val	GSS / telencephalic	Doh-ura, 1989[75] Hsiao, 1991[76]
145	Tyr - STOP	"GSS"	Kitamoto, 1993[77]
178	Asp - Asn	CJD / Fatal familial insomnia (FFI)	Goldfarb, 1991[78] Medori, 1992[7]
180	Val - Ile	CJD	Kitamoto, 1993[79]
198	Phe - Ser	GSS + NFTs*	Dlouhy, 1992[80]
200	Glu - Lys	CJD	Goldgaber, 1989[81]
208	Arg - His	CJD	Mastrianni, 1995[82]
210	Val - Ile	CJD	Pocchiari, 1993[83]
217	Gln - Arg	GSS + NFTs*	Hsiao, 1992[84]
232	Met - Arg	CJD	Kitamoto, 1993[79]
+48bp		CJD	Goldfarb, 1993[85]
+96bp	Extra insertions	CJD	Campbell, 1996[86]
+120bp	in the octapeptide	CJD	Goldfarb, 1991[87]
+144bp	repeat region (between codons 51 and 91)	CJD / ?	Owen, 1990[88] Collinge, 1992[89] Nicholl, 1995[90] Oda, 1995[91]
+168bp		CJD	Goldfarb, 1991[87]
+192bp		CJD / ?	Goldfarb, 1991[87] van Gool, 1995[92]
+216bp		CJD + ATD?**	Owen, 1992[93] Duchen, 1993[94]

*NFTs = Neurofibrillary tangles

**ATD = Alzheimer's disease

Legend for Table 5: Comparison of the ages of onset and disease durations of sporadic, familial and iatrogenic CJD cases studied here
Familial cases presented a decade earlier (comparison a), and had disease durations that were twice as long (comparison b).
The disease duration of iatrogenic cases was no different from sporadic ones (comparison d).

Table 5: Ages of onset and disease durations of sporadic, familial and iatrogenic CJD cases

	Age of onset (years)				Disease duration (months)			
	Mean	Minimum	Maximum	Median	Mean	Minimum	Maximum	Median
Sporadic	65[a],[c] (n = 143/144)	43	86	65	7[b],[d] (n = 139/144)	1	62	4
Familial	52[a] (n = 14/14)	35	67	55	19[b] (n = 12/14)	1	112	8
Iatrogenic	31[c] (n = 12/12)	20	46	29	7[d] (n = 11/12)	2	17	5

(a)	z - 4.1270	p < 0.0001	Corrected for ties
(b)	z - 2.2424	p = 0.0249	- do -
(c)	z - 5.6985	p < 0.0001	- do -
(d)	z - 1.7985	p = 0.0721	- do -

Table 6: Frequency of clinical signs at different stages of sporadic CJD

Clinical feature		At presentation	Early course	During illness
Cognitive -	Personality	14% (20)	51% (73)	53% (76)
	Behaviour	15% (22)	52% (74)	62% (89)
	Memory	19% (27)	57% (81)	64% (91)
	Disorientation	15% (21)	62% (88)	78% (112)
Dysphasia -	Expressive	4% (6)	27% (39)	38% (55)
	Receptive	0% (0)	5% (7)	20% (28)
Dyspraxia		3% (5)	44% (63)	55% (79)
Pyramidal dysfunction		3% (4)	20% (28)	62% (88)
Extra-pyramidal -	Parkinsonism	1% (1)	13% (18)	34% (48)
	Chorea	1% (1)	4% (6)	13% (18)
	Dystonia	0% (0)	6% (8)	16% (23)
Myoclonus		1% (1)	35% (50)	85% (122)
Epilepsia partialis continua		0% (0)	2% (3)	4% (6)
Visual -	blindness	9% (13)	35% (50)	52% (75)
	hallucinations	1% (2)	21% (30)	32% (46)
Cerebellar -	incoordination	39% (56)	76% (109)	85% (122)
	nystagmus	0% (0)	10% (15)	20% (29)
Oculomotor disturbance		1% (1)	14% (20)	27% (38)
Bulbar dysfunction		-	2% (3)	8% (12)
Muscle wasting		-	1% (2)	17% (24)
Incontinence		-	16% (23)	39% (56)
Primitive reflexes		-	-	58% (83)
Facial weakness		-	-	16% (23)
Paratonic rigidity		-	-	38% (55)
Seizures		-	-	13% (19)
Akinetic mutism		-	-	75% (107)
Respiratory depression		-	-	15% (22)

Legend for Table 7: The frequency of key clinical features in definite (n = 120) and probable (n = 23) sporadic CJD cases
Myoclonus, akinetic mutism and especially cortical blindness were more commonly recorded in the probable group.

Table 7:

Classification	Myoclonus	Cortical blindness	Akinetic mutism	Pyramidal signs (Pyr)	Extra-pyramidal signs (Par)	Cerebellar signs (C)	Pyr, Par or C
Definite	84% (101)	49% (59)	72% (87)	62% (74)	50% (60)	87% (104)	98% (117)
Probable	91% (21)	70% (16)	87% (20)	61% (14)	61% (14)	78% (18)	96% (22)

N.B. Data available in 120 definite and 23 probable cases.

Table 8: CJD of long duration

Reference	Age	Sex	Duration*	Clinical**	EEG	PRNP	Pathology
003	60	F	17	COB first (Heidenhain's)	Non-diag.	N/A	N/A
067	75	M	14	COG first "Classical"	Diag.	N/A	Diffuse spongiosis
105	65	F	13	CER first Cerebellar syn.	Non-diag.	129 MV	Diffuse spongiosis Plaques
109	77	F	14	CER first "Classical"	Diag.	N/A	Diffuse spongiosis
113	60	M	15	COG + CER "Classical"	Diag.	129 MM	Diffuse spongiosis
148	60	M	14	CER first "Classical"	Non-diag.	129 MM	Diffuse spongiosis
161	56	M	17	OTH first Parkinsonian	Diag.	N/A	Diffuse spongiosis
194	60	M	15	COG + CER "Classical"	Non-diag.	N/A	Diffuse spongiosis
201	53	F	17	CER first "Classical"	Diag.	129 MM	N/A
207	66	M	15	COG first "Classical"	Non-diag.	N/A	Diffuse spongiosis
221	63	F	12	CER first "Classical"	Non-diag.	N/A	N/A
225	71	M	22	OTH first "Classical"	Non-diag.	129 MM	N/A
316	57	M	13	COG first "Classical"	Non-diag.	N/A	N/A
339	65	M	12	CER first "Classical"	Non-diag.	N/A	N/A
251	51	M	62	COG first "Classical"	Non-diag.	N/A	Diffuse spongiosis
135	44	F	62	COG first "Classical"	Non-diag.	N/A	Diffuse spongiosis Plaques
237	72	F	28	COG first "Classical"	N/A	N/A	N/A
152	46	F	45	COG first "Classical"	N/A	N/A	N/A
159	74	M	30	OTH first "Classical"	Diag.	129 MM	N/A
282	59	F	26	COG first "Classical"	Non-diag.	N/A	N/A

N.B. *Duration of disease in months

**COG = cognitive dysfunction / CER = cerebellar incoordination / COB = cortical blindness / OTH = an alternative first clinical sign

N/A = information not available

Some of the cases have been pathologically confirmed as CJD in other centres

Table 9: Results of investigations in sporadic CJD cases

Investigation	Postive/abnormal	Negative/normal	No data	Percentage	
				Positive	Negative
EEG	61	72	11	46%	54%
Liver enzymes	58	64	22	48%	52%
CSF	32	54	58	37%	63%
CT/MRI	80	56	8	59%	41%

Table 10: EEG findings in cases with and without myoclonus

		EEG		
		Positive	**Negative**	**No data**
Myoclonus	**Present**	58	60	4
	Absent	3	12	6

Table 11: Cases of familial CJD

Reference	Site of PRNP mutation	Codon 129 genotype	Sex	Age at onset (years)	Duration (months)	Clinical diagnosis	Family history	EEG	Pathological diagnosis
046	178	MM	F	61	14	?CJD	Yes	Non-diag.	FFI
050	200	MM	F	67	1	CJD	Yes*	Diag.	CJD
056	+96 bpi**	MM	M	56	3	CJD	N/A	Diag.	CJD
122	200	MM	M	56	4	CJD	Yes	Diag.	CJD
145	102	MV	F	56	8	?CJD	Yes	Non-diag.	?GSS
165	N/A	N/A	M	66	20	CJD	Yes	N/A	N/A
172	200	MM	F	43	7	CJD	No	Diag.	CJD
173	+144 bpi**	MV	M	46	3	CJD	Yes	Diag.	CJD
210	102	MV	F	54	40	?CJD	Yes	Non-diag.	N/A
234	102	N/A	F	43	Alive	?ATD***	Yes	Non-diag.	-
219	178	MM	F	39	10	?CJD	No	Non-diag.	FFI
291	102	N/A	M	57	8	?ATD/?GSS	Yes	Non-diag.	GSS
301	+144 bpi**	N/A	M	35	112	?ATD	Yes	N/A	"Atypical prion dementia"[127,128]
309	117	N/A	F	43	Alive	GSS	Yes	Non-diag.	-

N.B. * Of Libyan Jewish extraction

** Extra base-pair insertions in the octapeptide repeat region

*** Alzheimer-type dementia

Table 12: Clinical characteristics of familial CJD (n = 14)

Clinical feature		At presentation	Early course	During illness
Cognitive -	Personality	21% (3)	36% (5)	43% (6)
	Behaviour	21% (3)	50% (7)	64% (9)
	Memory	14% (2)	43% (6)	64% (9)
	Disorientation	14% (2)	79% (11)	86% (12)
Dysphasia -	Expressive	7% (1)	43% (6)	64% (9)
	Receptive	0% (0)	7% (1)	36% (5)
Dyspraxia		0% (0)	36% (5)	71% (10)
Pyramidal dysfunction		0% (0)	7% (1)	36% (5)
Extra-pyramidal -	Parkinsonism	0% (0)	21% (3)	29% (4)
	Chorea	0% (0)	0% (0)	14% (2)
	Dystonia	0% (0)	0% (0)	14% (2)
Myoclonus		0% (0)	50% (7)	86% (12)
Epilepsia partialis continua		0% (0)	0% (0)	0% (0)
Visual -	blindness	7% (1)	21% (3)	36% (5)
	hallucinations	0% (0)	29% (4)	29% (4)
Cerebellar -	incoordination	21% (3)	71% (10)	86% (12)
	nystagmus	0% (0)	0% (0)	14% (2)
Oculomotor disturbance		0% (0)	21% (3)	36% (5)
Bulbar dysfunction		-	7% (1)	14% (2)
Muscle wasting		-	0% (0)	14% (2)
Incontinence		-	29% (4)	43% (6)
Primitive reflexes		-	-	71% (10)
Facial weakness		-	-	7% (1)
Paratonic rigidity		-	-	50% (7)
Seizures		-	-	14% (2)
Akinetic mutism		-	-	50% (7)
Respiratory depression		-	-	0% (0)

Legend for Table 13: Analysis by Spearman rank correlation of the clinical features of sporadic and familial CJD, at different stages of illness (see also Appendix 4)
At each of these points, the two groups show no differences.

Table 13: Rank correlation of clinical features, between sporadic and familial cases of CJD

Stage	**Rs**	**t**	**df**	**p**
At presentation	0.8695	7.042	16	<0.0001
Early	0.9032	9.172	19	<0.0001
During course	0.9043	10.592	25	<0.0001

N.B. All calculations corrected for ties

Table 14: Clinical characteristics of iatrogenic CJD with "peripheral" route of inoculation (n = 9)

Clinical feature		At presentation	Early course	During illness
Cognitive -	Personality	0% (0)	22% (2)	33% (3)
	Behaviour	0% (0)	0% (0)	11% (1)
	Memory	11% (1)	11% (1)	56% (5)
	Disorientation	0% (0)	0% (0)	22% (2)
Dysphasia -	Expressive	0% (0)	0% (0)	0% (0)
	Receptive	0% (0)	0% (0)	0% (0)
Dyspraxia		0% (0)	11% (1)	33% (3)
Pyramidal dysfunction		0% (0)	0% (0)	22% (2)
Extra-pyramidal -	Parkinsonism	0% (0)	0% (0)	11% (1)
	Chorea	0% (0)	11% (1)	11% (1)
	Dystonia	0% (0)	0% (0)	0% (0)
Myoclonus		0% (0)	44% (4)	78% (7)
Epilepsia partialis continua		0% (0)	0% (0)	0% (0)
Visual -	blindness	0% (0)	0% (0)	0% (0)
	hallucinations	0% (0)	0% (0)	0% (0)
Cerebellar -	incoordination	89% (8)	100% (9)	100% (9)
	nystagmus	0% (0)	33% (3)	56% (5)
Oculomotor disturbance		0% (0)	22% (2)	56% (5)
Bulbar dysfunction		-	0% (0)	11% (1)
Muscle wasting		-	11% (1)	11% (1)
Incontinence		-	22% (2)	44% (4)
Primitive reflexes		-	-	33% (3)
Facial weakness		-	-	11% (1)
Paratonic rigidity		-	-	0% (0)
Seizures		-	-	0% (0)
Akinetic mutism		-	-	44% (4)
Respiratory depression		-	-	0% (0)

Legend for Table 15: Analysis by Spearman rank correlation of the clinical features of sporadic and iatrogenic CJD via "peripheral" routes of inoculation, at different stages of illness (see also Appendix 4)
There is a significant difference in phenotype during early disease.

Table 15: Rank correlation of clinical features, between sporadic and iatrogenic cases of CJD with "peripheral" routes of inoculation

Stage	Rs	t	df	p
At presentation	0.5554	2.671	16	0.0167
Early	0.2117	0.944	19	0.3570
During course	0.6054	3.803	25	0.0008

N.B. All calculations corrected for ties

Table 16: Cases with (pathologically confirmed) non-CJD

Diagnosis	Frequency
Alzheimer-type dementia (ATD)	17
ATD + Multi-infarct disease (MID)	5
MID	3
Diffuse Lewy body disease (DLBD)	1
ATD + DLBD	1
Motor neurone disease	2
Cerebrovascular disease	1
Cerebellar degeneration	1
Pick's disease	1
Progressive supranuclear palsy	1
Multiple system atrophy	1
Cortico-basal degeneration	1
Viral encephalomyelitis	1
Metastatic carcinoma	1
Hypoxia	1
Epilepsy	1
Spongiform myelinopathy	1
No abnormality found	1

Table 17: Comparison of clinical features at presentation, between CJD and non-CJD cases

Clinical feature		CJD	Non-CJD	Sensitivity	Specificity	+ve PV	-ve PV
Cognitive -	Personality	23	4	0.1465	0.8889	0.8519	0.1928
	Behaviour	25	1	0.1592	0.9722	0.9615	0.2096
	Memory	29	9	0.1847	0.7500	0.7632	0.1742
	Disorientation	23	11	0.1465	0.6944	0.6765	0.1572
Dysphasia -	Expressive	7	2	0.0446	0.9444	0.7778	0.1848
	Receptive	0	0	0.0000	1.0000	-	0.1865
Dyspraxia		5	1	0.0318	0.9722	0.8333	0.1872
Pyramidal dysfunction		4	1	0.0255	0.9722	0.8000	0.1862
Extra-pyramidal -	Parkinsonism	1	1	0.0064	0.9722	0.5000	0.1832
	Chorea	1	0	0.0064	1.0000	1.0000	0.1875
	Dystonia	0	0	0.0000	1.0000	-	0.1865
Myoclonus		1	0	0.0064	1.0000	1.0000	0.1875
Epilepsia partialis continua		0	0	0.0000	1.0000	-	0.1865
Visual -	blindness	14	1	0.0892	0.9722	0.9333	0.1966
	hallucinations	2	1	0.0127	0.9722	0.6667	0.1842
Cerebellar -	incoordination	59	2	0.3758	0.9444	0.9672	0.2576
	nystagmus	0	0	0.0000	1.0000	-	0.1865
Oculomotor disturbance		1	1	0.0064	0.9722	0.5000	0.1832

Table 18: Comparison of clinical features during the early phase of illness, between CJD and non-CJD cases

Clinical feature		CJD	Non-CJD	Sensitivity	Specificity	+ve PV	-ve PV
Cognitive -	Personality	78	11	0.4968	0.6944	0.8764	0.2404
	Behaviour	81	13	0.5159	0.6389	0.8617	0.2323
	Memory	87	20	0.5541	0.4444	0.8131	0.1860
	Disorientation	99	23	0.6306	0.3611	0.8115	0.1831
Dysphasia -	Expressive	45	4	0.2866	0.8889	0.9184	0.2222
	Receptive	8	2	0.0510	0.9444	0.8000	0.1858
Dyspraxia		68	19	0.4331	0.4722	0.7816	0.1604
Pyramidal dysfunction		29	8	0.1847	0.7778	0.7838	0.1795
Extra-pyramidal -	Parkinsonism	21	7	0.1338	0.8056	0.7500	0.1758
	Chorea	6	0	0.0382	1.0000	1.0000	0.1925
	Dystonia	8	0	0.0510	1.0000	1.0000	0.1946
Myoclonus		57	10	0.3631	0.7222	0.8507	0.2063
Epilepsia partialis continua		3	0	0.0191	1.0000	1.0000	0.1895
Visual -	blindness	53	5	0.3376	0.8611	0.9138	0.2296
	hallucinations	34	6	0.2166	0.8333	0.8500	0.1961
Cerebellar -	incoordination	119	8	0.7580	0.7778	0.9370	0.4242
	nystagmus	15	1	0.0955	0.9722	0.9375	0.1977
Oculomotor disturbance		23	1	0.1465	0.9722	0.9583	0.2071
Bulbar dysfunction		4	1	0.0255	0.9722	0.8000	0.1862
Muscle wasting		2	2	0.0127	0.9444	0.5000	0.1799
Incontinence		27	11	0.1720	0.6944	0.7105	0.1613

Table 19: Comparison of clinical features during the course of illness, between CJD and non-CJD cases

Clinical feature		CJD	Non-CJD	Sensitivity	Specificity	+ve PV	-ve PV
Cognitive -	Personality	82	13	0.5223	0.6389	0.8632	0.2347
	Behaviour	98	18	0.6242	0.5000	0.8448	0.2338
	Memory	100	21	0.6369	0.4117	0.8264	0.2083
	Disorientation	124	27	0.7898	0.2500	0.8212	0.2143
Dysphasia -	Expressive	64	12	0.4076	0.6667	0.8421	0.2051
	Receptive	33	5	0.2102	0.8611	0.8684	0.2000
Dyspraxia		89	19	0.5669	0.4722	0.8241	0.2000
Pyramidal dysfunction		93	20	0.5924	0.4444	0.8230	0.2000
Extra-pyramidal -	Parkinsonism	52	11	0.3312	0.6944	0.8254	0.1923
	Chorea	20	2	0.1274	0.9444	0.9091	0.1988
	Dystonia	25	1	0.1592	0.9722	0.9615	0.2096
Myoclonus		134	20	0.8535	0.4444	0.8701	0.4103
Epilepsia partialis continua		6	0	0.0382	1.0000	1.0000	0.1925
Visual -	blindness	80	6	0.5096	0.8333	0.9302	0.2804
	hallucinations	50	6	0.3185	0.8333	0.8929	0.2190
Cerebellar -	incoordination	134	14	0.8535	0.6111	0.9054	0.4889
	nystagmus	31	1	0.1975	0.9722	0.9688	0.2174
Oculomotor disturbance		43	4	0.2739	0.8889	0.9149	0.2192
Bulbar dysfunction		14	3	0.0892	0.9167	0.8235	0.1875
Muscle wasting		26	6	0.1656	0.8333	0.8125	0.1863
Incontinence		62	14	0.3949	0.6111	0.8158	0.1880
Primitive reflexes		93	16	0.5924	0.5556	0.8532	0.2381
Facial weakness		24	5	0.1529	0.8611	0.8276	0.1890
Paratonic rigidity		62	10	0.3949	0.7222	0.8611	0.2149
Seizures		21	6	0.1338	0.8333	0.7778	0.1807
Akinetic mutism		114	8	0.7261	0.7778	0.9344	0.3944
Respiratory depression		22	7	0.1401	0.8056	0.7586	0.1768

Table 20: Comparison of clinical features between pathologically confirmed (other) and pathologically unconfirmed (other#) cases of non-CJD, at presentation

Clinical feature		Non-CJD + PM (n = 36)		Non-CJD + No PM (n = 30)	
Cognitive -	Personality	4	(11%)	7	(23%)
	Behaviour	1	(3%)	2	(7%)
	Memory	9	(25%)	15	(50%)
	Disorientation	11	(31%)	10	(33%)
Dysphasia -	Expressive	2	(6%)	2	(7%)
	Receptive	0	(0%)	0	(0%)
Dyspraxia		1	(3%)	0	(0%)
Pyramidal dysfunction		1	(3%)	0	(0%)
Extra-pyramidal -	Parkinsonism	1	(3%)	2	(7%)
	Chorea	0	(0%)	1	(3%)
	Dystonia	0	(0%)	0	(0%)
Myoclonus		0	(0%)	0	(0%)
Epilepsia partialis continua		0	(0%)	0	(0%)
Visual -	blindness	1	(3%)	0	(0%)
	hallucinations	1	(3%)	2	(7%)
Cerebellar -	incoordination	2	(6%)	7	(23%)
	nystagmus	0	(0%)	0	(0%)
Oculomotor disturbance		1	(3%)	0	(0%)

Table 21: Comparison of clinical features between pathologically confirmed (other) and pathologically unconfirmed (other#) cases of non-CJD, early in the course

Clinical feature		Non-CJD + PM (n = 36)		Non-CJD + no PM (n = 30)	
Cognitive -	Personality	11	(31%)	9	(30%)
	Behaviour	13	(36%)	8	(27%)
	Memory	20	(56%)	19	(63%)
	Disorientation	23	(64%)	16	(53%)
Dysphasia -	Expressive	4	(11%)	5	(17%)
	Receptive	2	(6%)	1	(3%)
Dyspraxia		19	(53%)	9	(30%)
Pyramidal dysfunction*		8	(22%)	2	(7%)
Extra-pyramidal -	Parkinsonism	7	(19%)	2	(7%)
	Chorea	0	(0%)	3	(10%)
	Dystonia	0	(0%)	0	(0%)
Myoclonus		10	(28%)	15	(50%)
Epilepsia partialis continua		0	(0%)	0	(0%)
Visual -	blindness**	5	(14%)	0	(0%)
	hallucinations	6	(17%)	6	(20%)
Cerebellar -	incoordination	8	(22%)	10	(33%)
	nystagmus	1	(3%)	1	(3%)
Oculomotor disturbance		1	(3%)	0	(0%)
Bulbar dysfunction		1	(3%)	0	(0%)
Muscle wasting		2	(6%)	0	(0%)
Incontinence***		11	(31%)	0	(0%)

* RR = 1.60, 95% CIs = 1.07 - 2.40
** RR = 1.97, 95% CIs = 1.54 - 2.52
*** RR = 2.20, 95% CIs = 1.65 - 2.94

Table 22: Comparison of clinical features between pathologically confirmed (other) and pathologically unconfirmed (other#) cases of non-CJD, during illness

Clinical feature		Non-CJD + PM (n = 36)		Non-CJD + no PM (n = 30)	
Cognitive -	Personality	13	(36%)	10	(33%)
	Behaviour	18	(50%)	13	(43%)
	Memory	21	(58%)	22	(73%)
	Disorientation	27	(75%)	19	(63%)
Dysphasia -	Expressive	12	(33%)	10	(33%)
	Receptive	5	(14%)	5	(17%)
Dyspraxia		19	(53%)	14	(47%)
Pyramidal dysfunction		20	(56%)	13	(43%)
Extra-pyramidal -	Parkinsonism	11	(31%)	5	(17%)
	Chorea	2	(6%)	6	(20%)
	Dystonia	1	(3%)	0	(0%)
Myoclonus		20	(56%)	20	(67%)
Epilepsia partialis continua		0	(0%)	0	(0%)
Visual -	blindness	6	(17%)	2	(7%)
	hallucinations	6	(17%)	7	(23%)
Cerebellar -	incoordination	14	(39%)	13	(43%)
	nystagmus	1	(3%)	1	(3%)
Oculomotor disturbance		4	(11%)	0	(0%)
Bulbar dysfunction		3	(8%)	0	(0%)
Muscle wasting		6	(17%)	3	(1%)
Incontinence		14	(39%)	6	(2%)
Primitive reflexes		16	(44%)	10	(33%)
Facial weakness*		5	(14%)	1	(3%)
Paratonic rigidity		10	(28%)	7	(23%)
Seizures		6	(17%)	6	(20%)
Akinetic mutism**		8	(22%)	1	(3%)
Respiratory depression***		7	(19%)	0	(0%)

*RR = 1.61, 95% CIs = 1.05 - 2.49 **RR = 1.81, 95% CIs = 1.27 - 2.57 ***RR = 2.03, 95% CIs = 1.57 - 2.64

Table 23: Comparison of clinical and pathological features in different areas of CJD brain

Area	Clinical	Pathology		Chi square	Significance*
		Sponge +	Sponge -		
Frontal	Signs +	43	15	7.09	0.0077
	Signs -	5	10		
Temporal	Signs +	43	8	0.15	0.7031
	Signs -	20	2		
Parietal	Signs +	32	16	0.00	0.9744
	Signs -	16	9		
Basal ganglia	Signs +	30	4	1.18	0.2766
	Signs -	38	1		
Occipital	Signs +	31	8	0.00	0.9902
	Signs -	28	6		
Cerebellar	Signs +	58	3	3.03	0.0818
	Signs -	9	3		
Brainstem	Signs +	1	7	0.31	0.5794
	Signs -	8	57		

* All calculations with df = 1 and Yates' correction

Table 24: Sporadic CJD cases with anti-PrP positive amyloid plaques

Case	Age	Sex	Duration	Clinical*	EEG**	Sponge***	Codon 129 genotype**
105	65	F	13	CER presentation; cerebellar syndrome	Non-diag.	Frontal/Temporal/BG/ Occipital/Cerebellar	MV
135	44	F	62	COG presentation; classical but "long duration"	Non-diag.	Frontal/Temporal/Parietal/ BG/Occipital/Cerebellar	N/A
229	61	F	7	COG presentation; classical	Non-diag.	Parietal/BG/Occipital/ Cerebellar	N/A
227	60	M	4	CER presentation; classical	Non-diag.	Frontal/Temporal/Parietal/ BG/Occipital/Cerebellar	VV
244	63	F	9	CER presentation; classical	Non-diag.	Frontal/Temporal/Parietal/ BG/Occipital/Cerebellar	MV
265	75	M	3	CER presentation; classical	N/A	Temporal/BG/Occipital/ Cerebellar/Brainstem	N/A
257	56	F	3	COG presentation; classical	Diagnostic	Frontal/Temporal/Parietal/ Occipital/Brainstem	MM
304	76	F	5	COG presentation; classical	Diagnostic	Frontal/Temporal/Parietal/ Occipital/Cerebellar	N/A
283	78	F	4	COG presentation; classical	Non-diag.	Frontal/Temporal/BG/ Occipital/Cerebellar	N/A
147	65	M	2	CER presentation; classical	Non-diag.	Frontal/Temporal/Parietal/ BG/Occipital/Cerebellar	N/A
305	75	F	4	CER presentation; classical	Non-diag.	Frontal/Temporal/Parietal/ BG/Cerebellar	N/A

N.B. *CER = cerebellar / COG = cognitive dysfunction

**N/A = data not available

***BG = basal ganglia

Table 25: Comparisons of the ages of onset and disease durations between familial CJD cases, homozygous and heterozygous with respect to the common polymorphism at codon 129

Codon 129 genotype	No.	Age at onset (yrs)			Disease duration (mnths)		
		Mean	Range	Median	Mean	Range	Median
MM	6	54*	39 - 67	56	7**	1 - 14	6
MV	3	52*	46 - 56	54	17**	3 - 40	8
VV	0	-	-	-	-	-	-

* Mann Whitney U = 7.0; not significant

** Mann Whitney U = 6.5; not significant

Table 26: Comparison of ages of onset and disease durations between sporadic CJD cases with different codon 129 genotypes

Genotype	Age (years)				Duration (months)			
	No.	Mean	Range	Median	No.	Mean	Range	Median
MM	57	66	49 - 86	66	55	5	1 - 30	3
MV	6	67	61 - 79	65	6	6	2 - 13	4
VV	5	58	48 - 63	59	5	5	1 - 10	4

Table 27: Clinical and pathological features of sporadic CJD cases with Met/Val or Val/Val encoded by codon 129 of PRNP ORF

Case	Codon 129 genotype	Clinical features*	EEG	Pathology**
57	MV	CER first; then classical	Non-diag.	Temporal, parietal and cerebellar sponge
105	MV	CER first; progressive cerebellar syndrome	Non-diag.	Frontal, temporal, başal ganglia, occipital and cerebellar sponge. Anti-PrP positive plaques
115	MV	Pyramidal dysfunction first; then classical	Non-diag.	Frontal, temporal, parietal, basal ganglia, occipital, cerebellar and brainstem sponge
162	MV	CER first; then classical	Diagnostic	N/A
244	MV	CER first; then classical	Non-diag.	Frontal, temporal, parietal, basal ganglia, occipital and cerebellar sponge. Anti-PrP positive plaques
250	MV	CER with cortical deafness at start; then classical	Non-diag.	Frontal, temporal, basal ganglia, occipital and cerebellar sponge
27	VV	CER first; then classical	Non-diag.	N/A
97	VV	COG and CER dysfunction at start; then classical	Non-diag.	Temporal, basal ganglia, occipital and cerebellar sponge
125	VV	CER first; then classical	Non-diag.	Frontal, temporal, parietal, basal ganglia, occipital, cerebellar and brainstem sponge
149	VV	CER first; then classical	Non-diag.	Frontal, temporal, parietal, basal ganglia, occipital and cerebellar sponge
227	VV	CER first; then classical	Non-diag.	Frontal, temporal, parietal, basal ganglia, occipital and cerebellar sponge. Anti-PrP positive plaques

NB *CER = cerebellar / COG = cognitive dysfunction as first features

**N/A = data unavailable

Table 28: Data on genetic information requested after counselling

Case no.	Result requested?	Family history?	Respondent	University education?
240	Yes	Possible	Spouse	Yes
242	Yes	Possible	Offspring	No
272	Yes	No	Sibling	No
287	Yes	Possible	Offspring	No
285	Yes	No	Spouse	No
305	Yes	No	Offspring	No
275	No	No	Spouse	Yes
276	Yes	No	Spouse	No
243	Yes	Possible	Offspring	No
245	Yes	No	Offspring	No
277	Yes	Possible	Spouse	No
250	Yes	No	Spouse	No
248	Yes	No	Spouse	No
259	No	No	Spouse	No
244	Yes	No	Spouse	No
281	Yes	No	Spouse	No
268	No	Possible	Spouse	Yes
317	Yes	No	Offspring	No
302	No	Possible	Offspring	No
280	Yes	No	Sibling	No
289	Yes	No	Offspring	No
267	Yes	No	Spouse	No
288	Yes	No	Spouse	No
257	No	Possible	Spouse	No
309	Yes	Definite	Spouse	No
261	No	No	Spouse	No
256	No	No	Spouse	No
262	No	Possible	Spouse	No
283	Yes	Possible	Spouse	No
292	Yes	No	Spouse	No

FIGURES

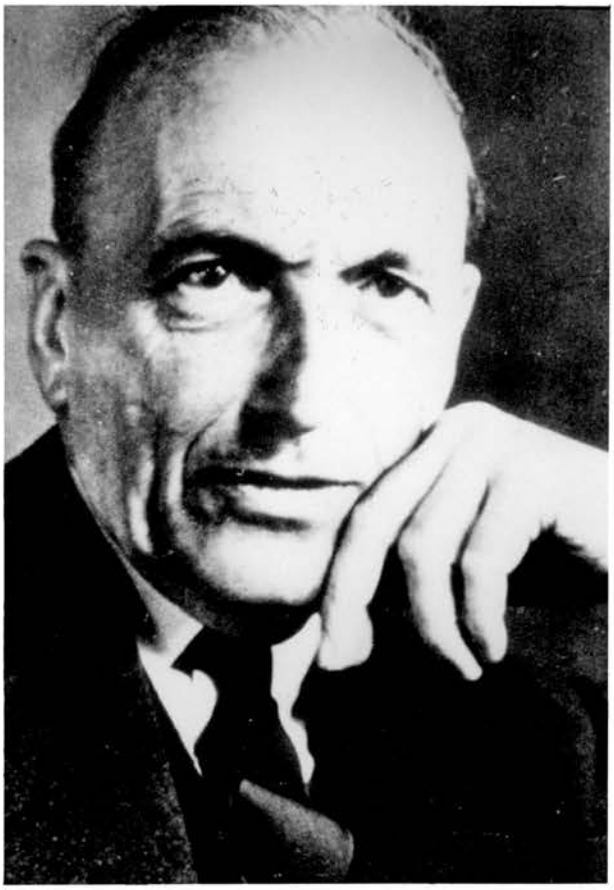


Figure 1: H.G. Creutzfeldt (b 1883)

Figure 2: A. Jakob (1884 - 1931)

Legend for Figure 3: Diagram illustrating the proposed interaction of nascent, protease-sensitive prion protein (blue boxes, PrP^{C}) with infective, protease-resistant prion protein (red balls, PrP^{Sc}). The theory holds that the process is auto-catalytic, therefore converting more PrP^{C} to PrP^{Sc}.

Figure 3: Interaction of PrP^{C} with PrP^{Sc}, and its conversion to PrP^{Sc}

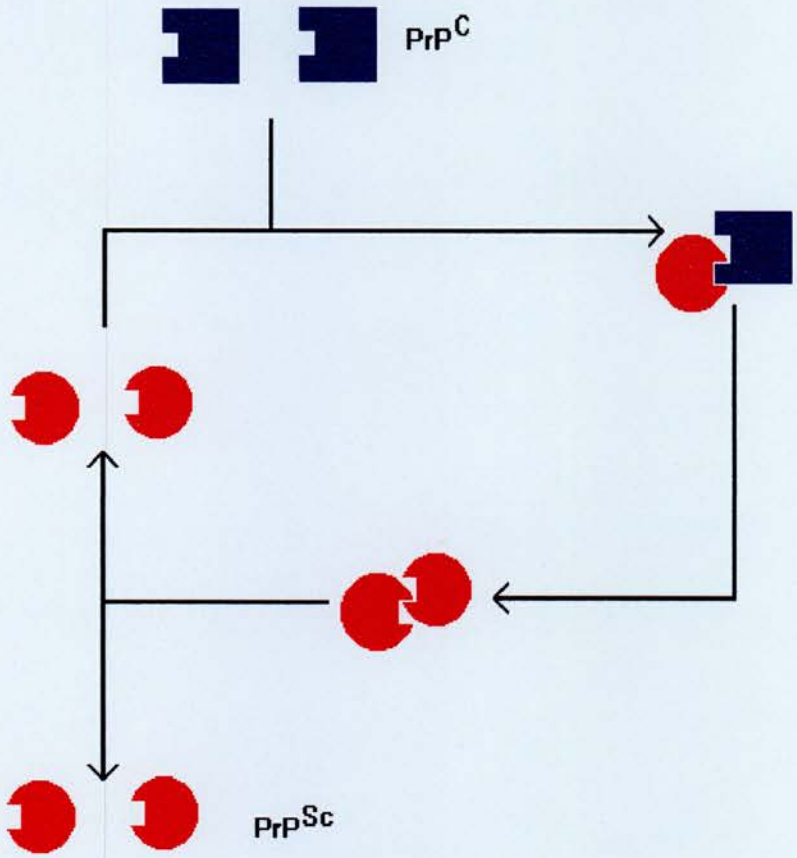
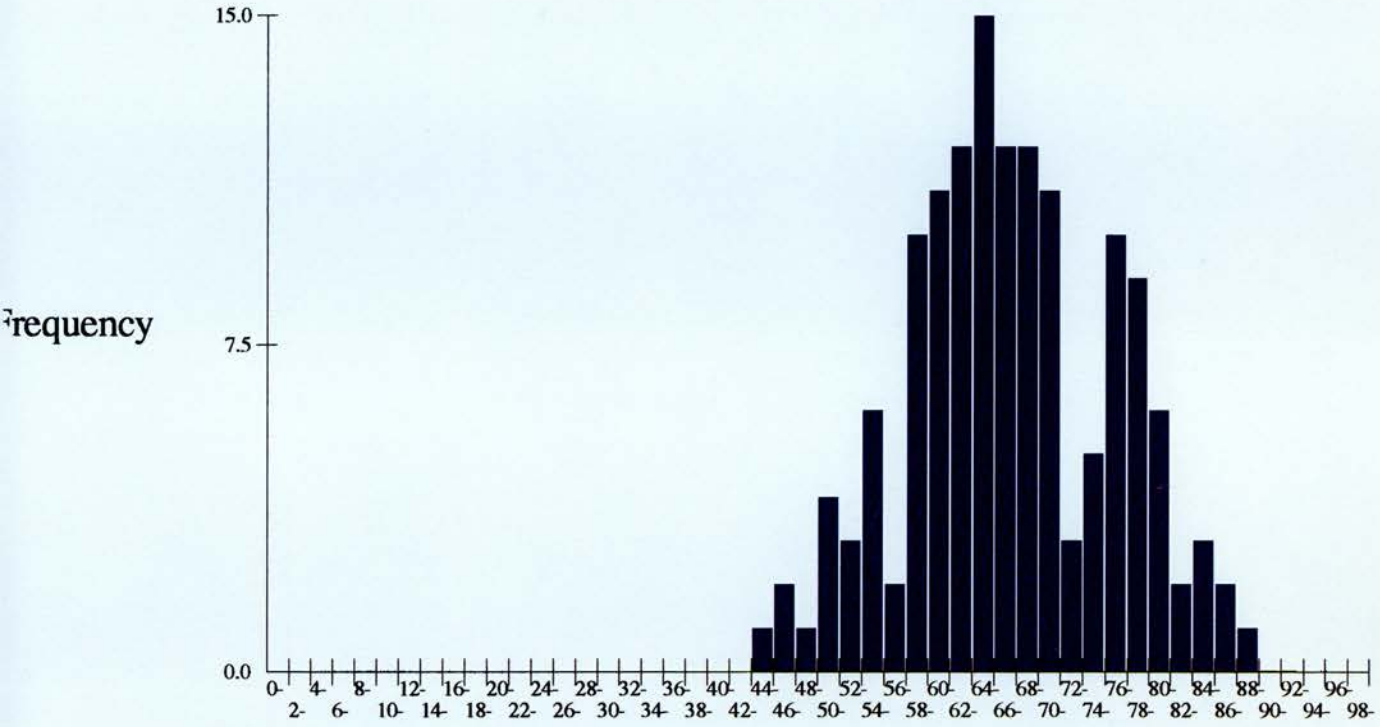


Figure 4: Age distribution of sporadic CJD cases



Legend for Figure 5: Correlation of disease duration with age of onset in sporadic CJD

There is a significant negative correlation between these modalities (r - 0.2822, p = 0.0008).

Figure 5: Correlation of disease duration with age of onset in sporadic CJD

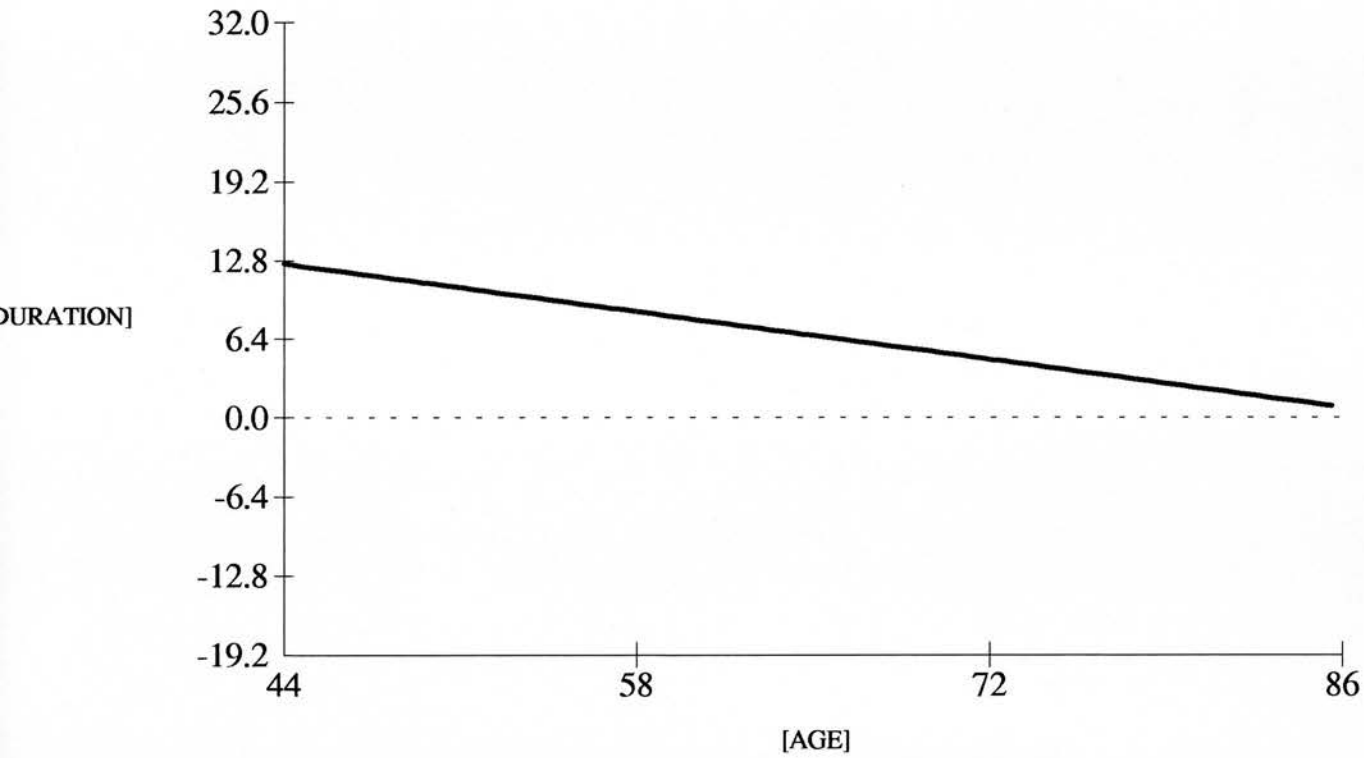


Figure 6: Disease duration (in months) in sporadic CJD

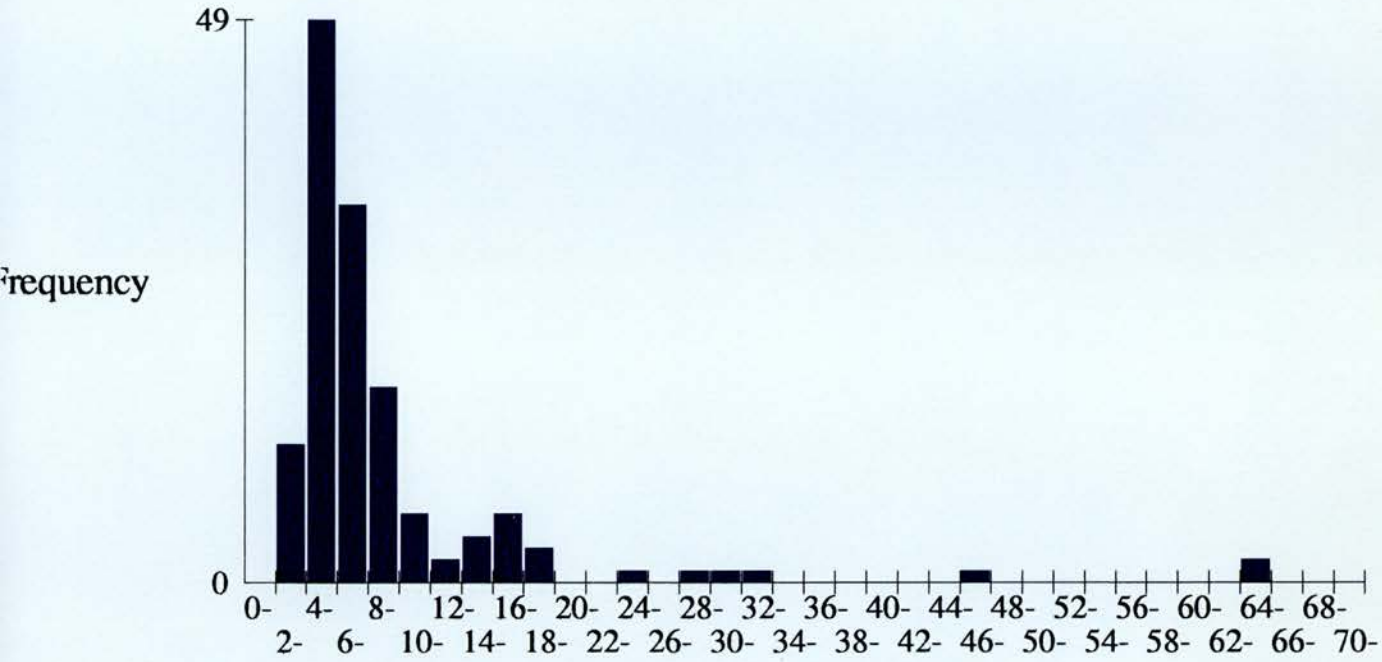


Figure 7: EEG appearances considered "typical" of CJD

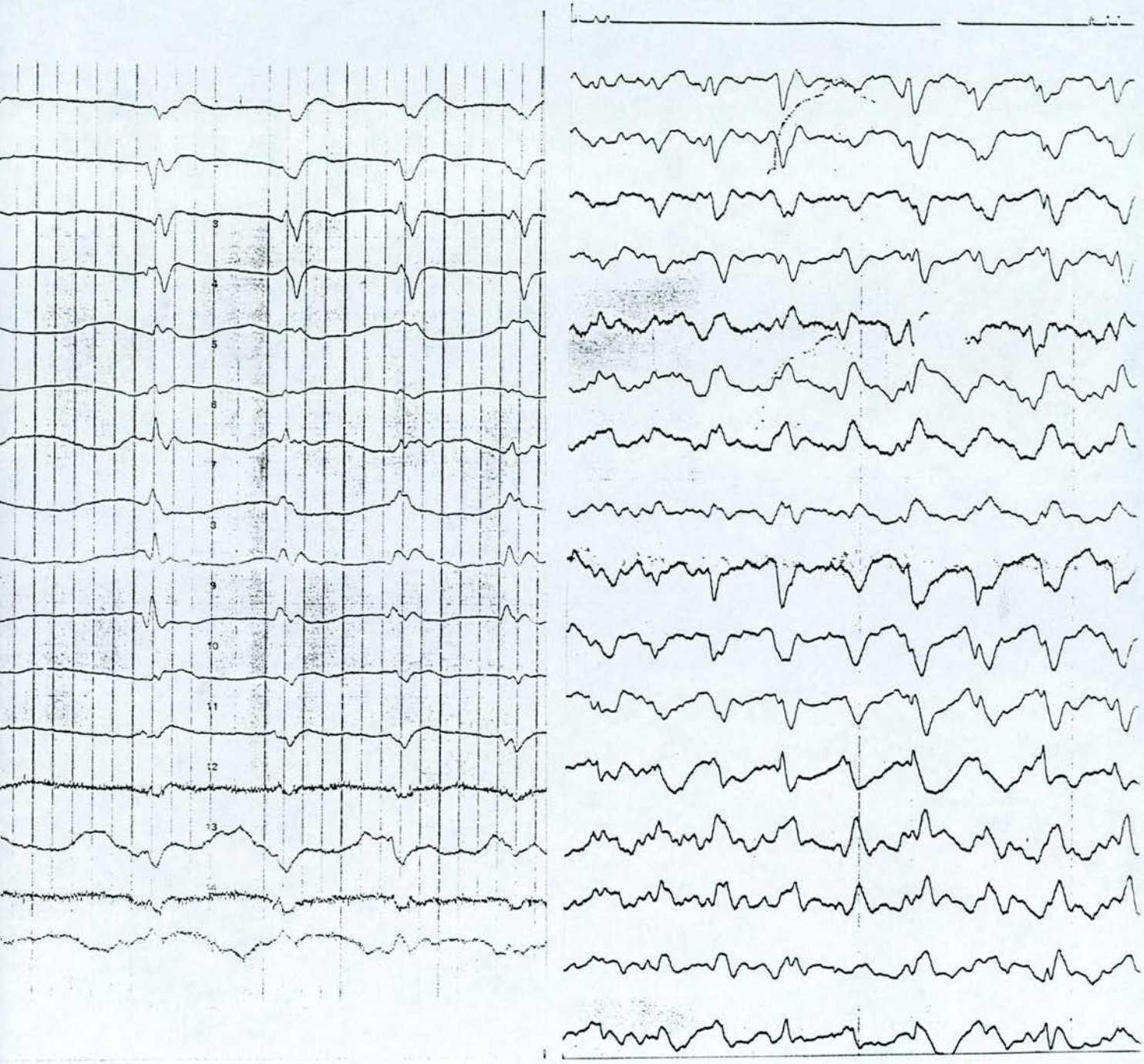
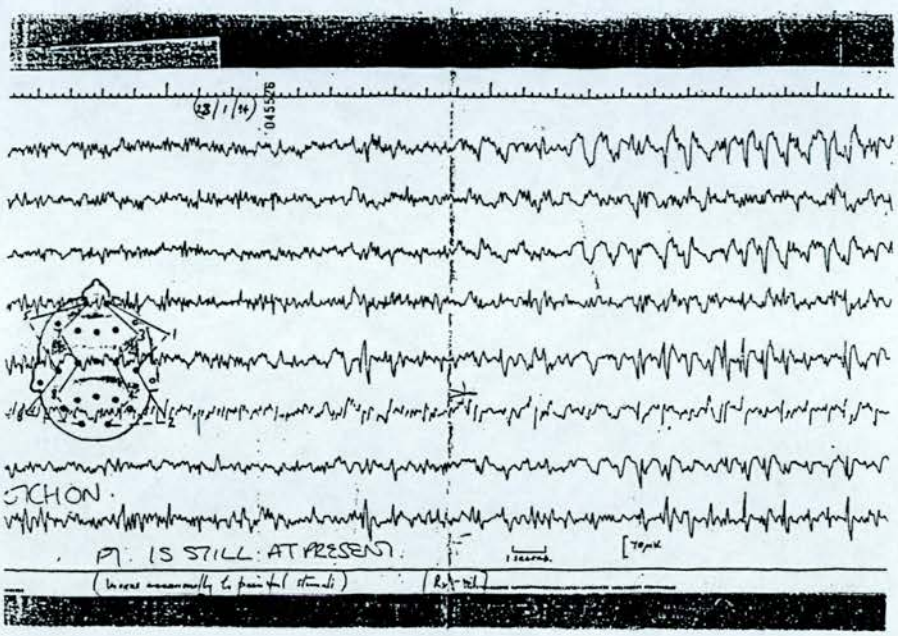


Figure 8: Two EEGs from (pathologically confirmed) case of CJD, performed 6 days apart



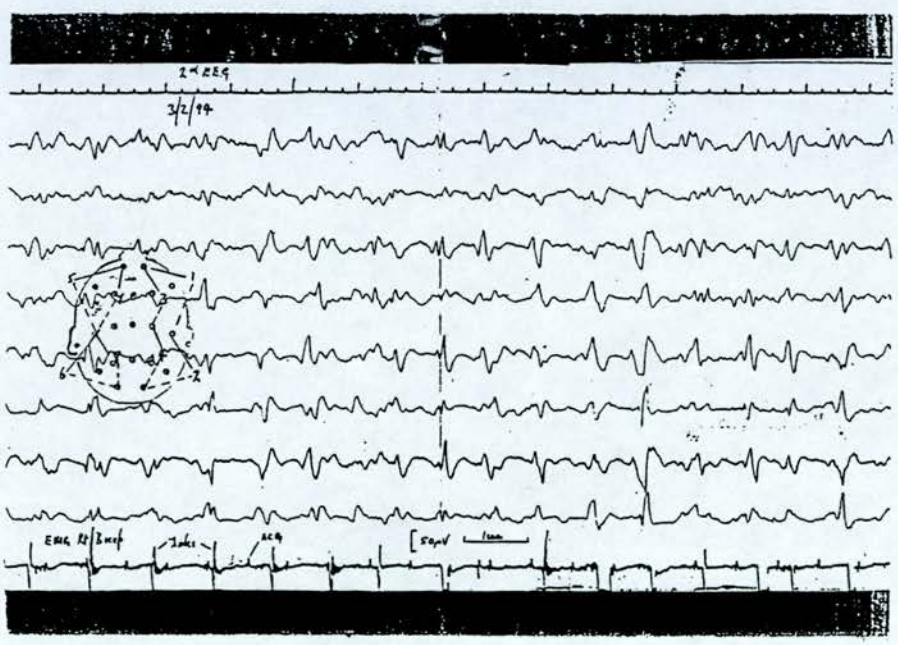


Figure 9: EEG appearance considered atypical

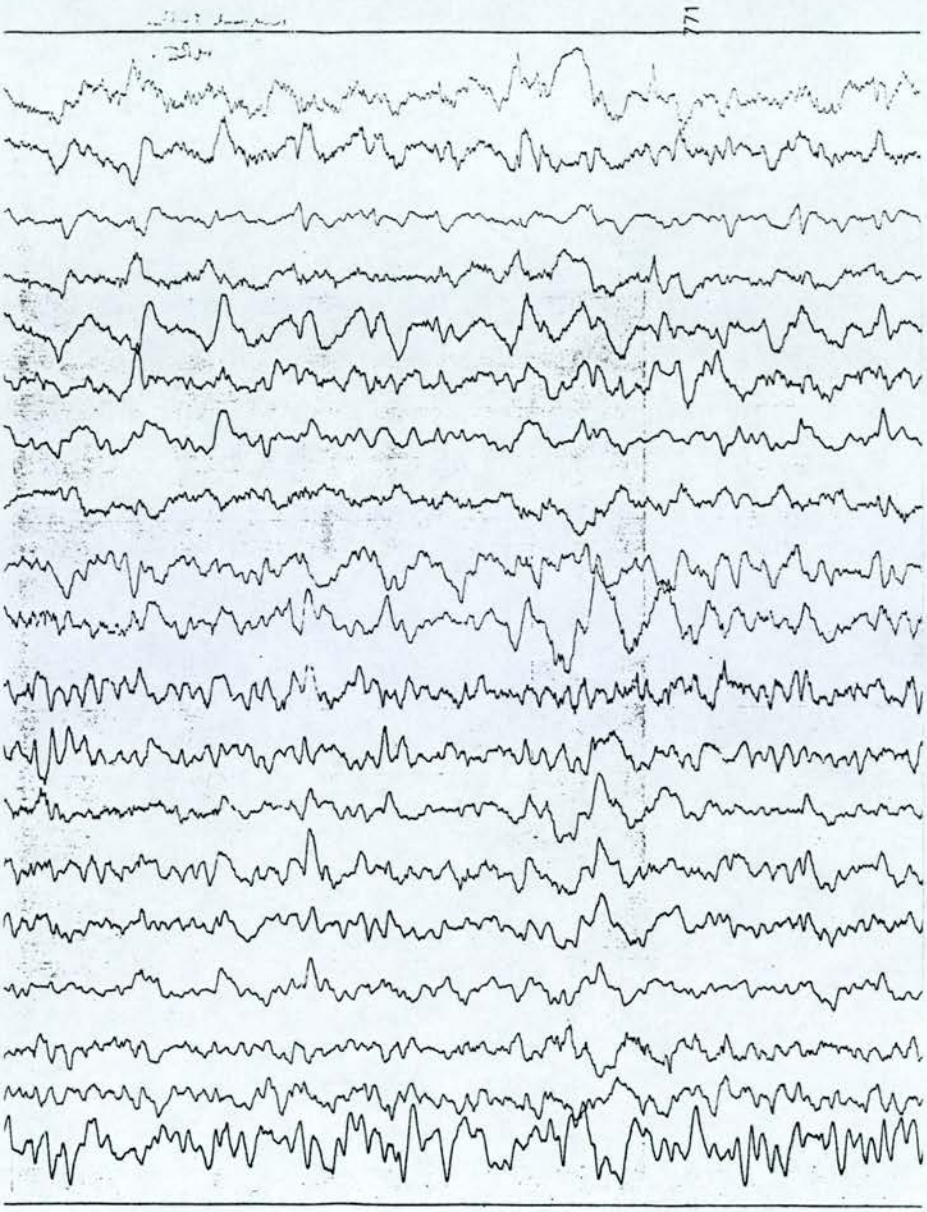


Figure 10: "Suggestive" EEG appearances in patient with (pathologically confirmed) Alzheimer's disease

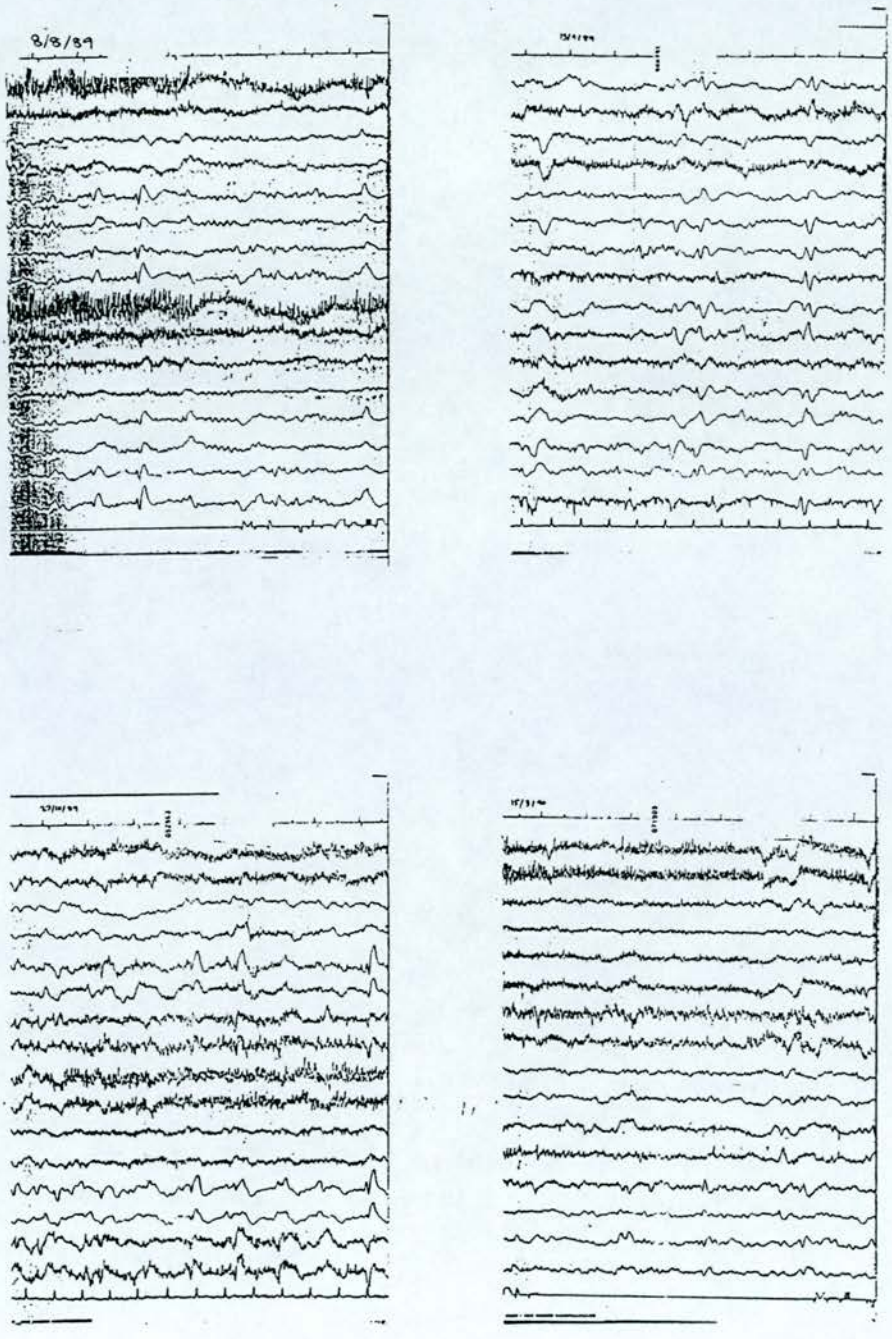
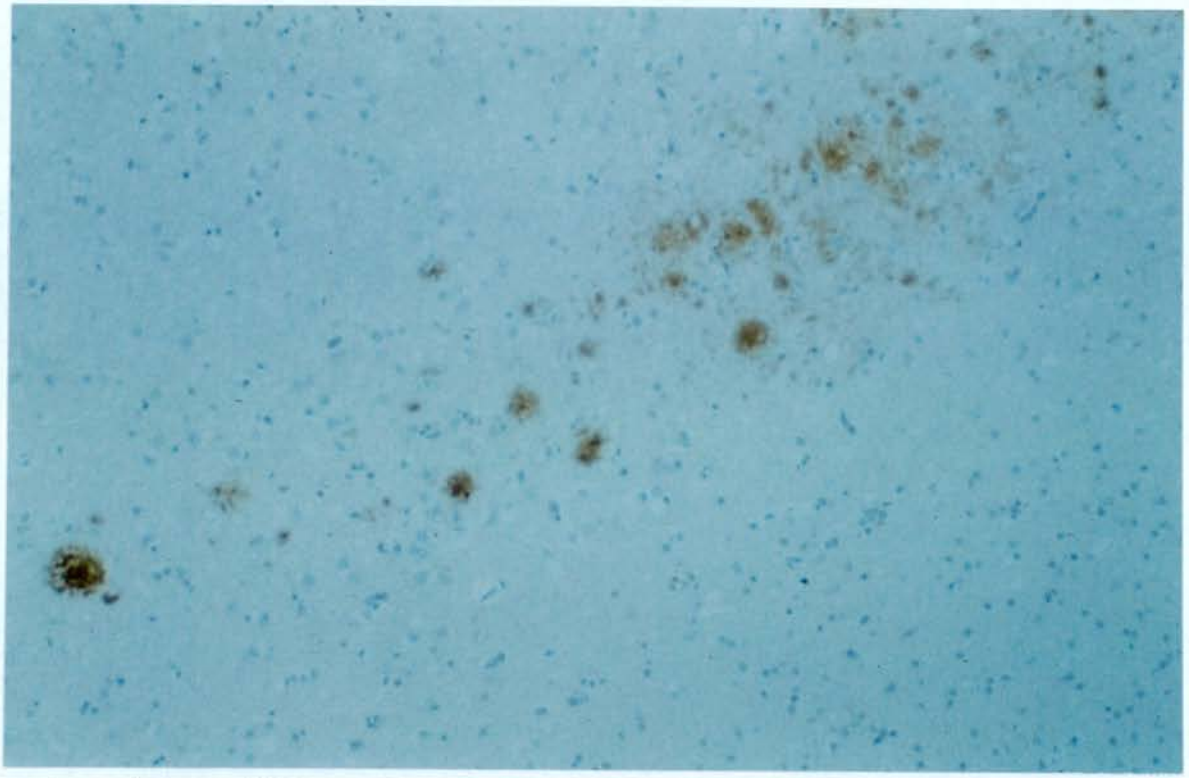


Figure 11: Serial liver enzyme measurements in definite case of CJD (ref. no. 250)

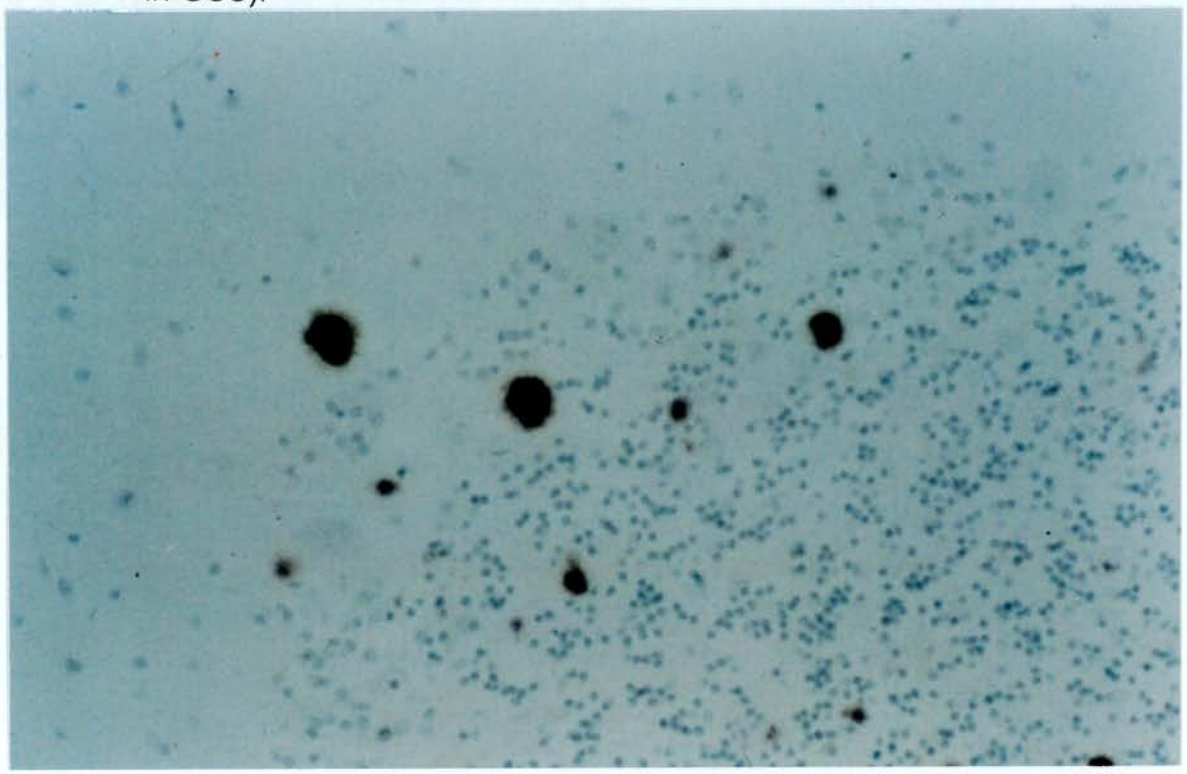
Withdrawal		Sod. 135 - 145	Pot. 3.5 - 5.0	Chlor. 101 - 111	Tot.CO_2 23 - 30	Urea 2.5 - 7.5	Creat. 40 - 130 µmol/l	H+ 36 - 43 nmol/l	Pco_2 4.6 - 6.0	Po_2 10.5 - 13.5	Bicarb. 23 - 30 mmol/l	Glucose 3.3 - 5.6 mmol/l Fasting	Pl.Osm. 275 - 295	Urine Osmol.	Bili (Paed.) µmol/l	
Date	Time	mmol/l							kPa				mmol/kg			
05.05.93	16:00	141	4.0	104	25	7.5	113					7.6				
11.05.93	13:00	142	3.3	106	27	5.9	84					9.8				
21.05.93	13:30	137	3.9	105	22	4.2	75					8.4				
25.05.93	13:00	137	4.2	108	23	5.4	84					6.8				
28.05.93	16:00	138	4.0	105	23	4.7	73					8.9				K

Withdrawal		Calc. 2.12 - 2.62	Phos. 0.70 - 1.40	T. Prot. 62 - 82	Alb. 35 - 50	Glob. 20 - 35	Bili. 5 - 20 (µmol/l)	Alk. Phos. 70 - 350	AaT. <40	AlT <40	LDH 240 - 525	CK <170	GGT M <50 F <35	
Date	Time	mmol/l		g/l				IU/l						
05.05.93	16:00	2.43	1.03	68	41	27	13	290	45	42			350	
11.05.93	13:00			74	45	29	15	337	45	43			349	
21.05.93	13:30	2.16	0.99	64	32	32	37	599	62	44			222	
25.05.93	13:00													
28.05.93	16:00	2.16	0.95	65	29	36	61	463	52	34			143	K

Figure 12: Amyloid plaques in CJD. Immunocytochemistry for prion protein is demonstrated (avidin-biotin reaction after hydrolytic autoclaving and hematoxylin counterstain, originally x 240).



a) In case 145, carrying Pro - Leu mutation at codon 102. Multicentric plaques are present, but they are not large and confluent (as seen in GSS).



b) In case of sporadic CJD. The plaques are unicentric.

Legend for Figure 13: The polymorphic status at codon 129 in cases (blue columns) and non-cases (green columns) of CJD

In tabular form the data is as follows:

Codon 129 genotype	CJD	Non-CJD
MM	57	8
MV	6	4
VV	5	4

Chi squared = 8.527, df = 2, p = 0.0141.

Figure 13: Comparison of codon 129 genotype

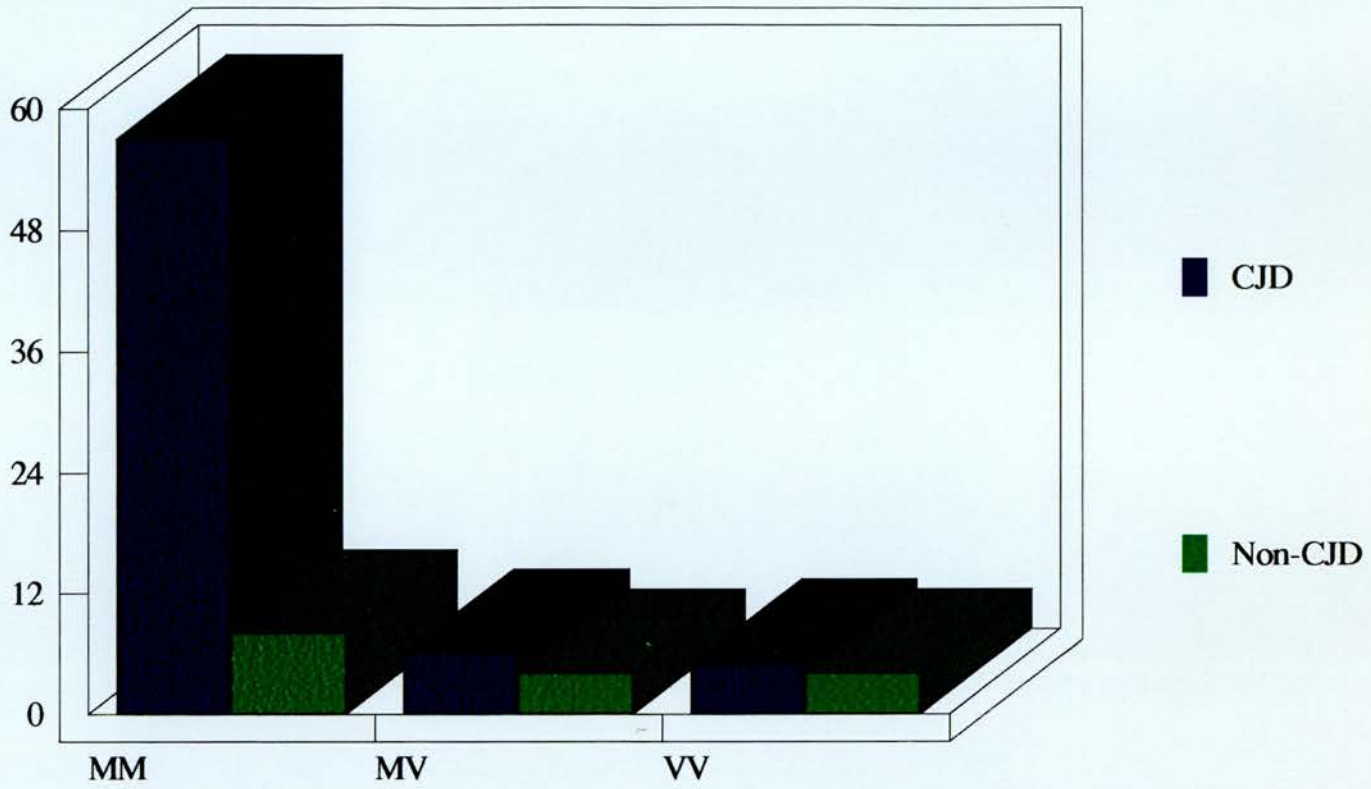
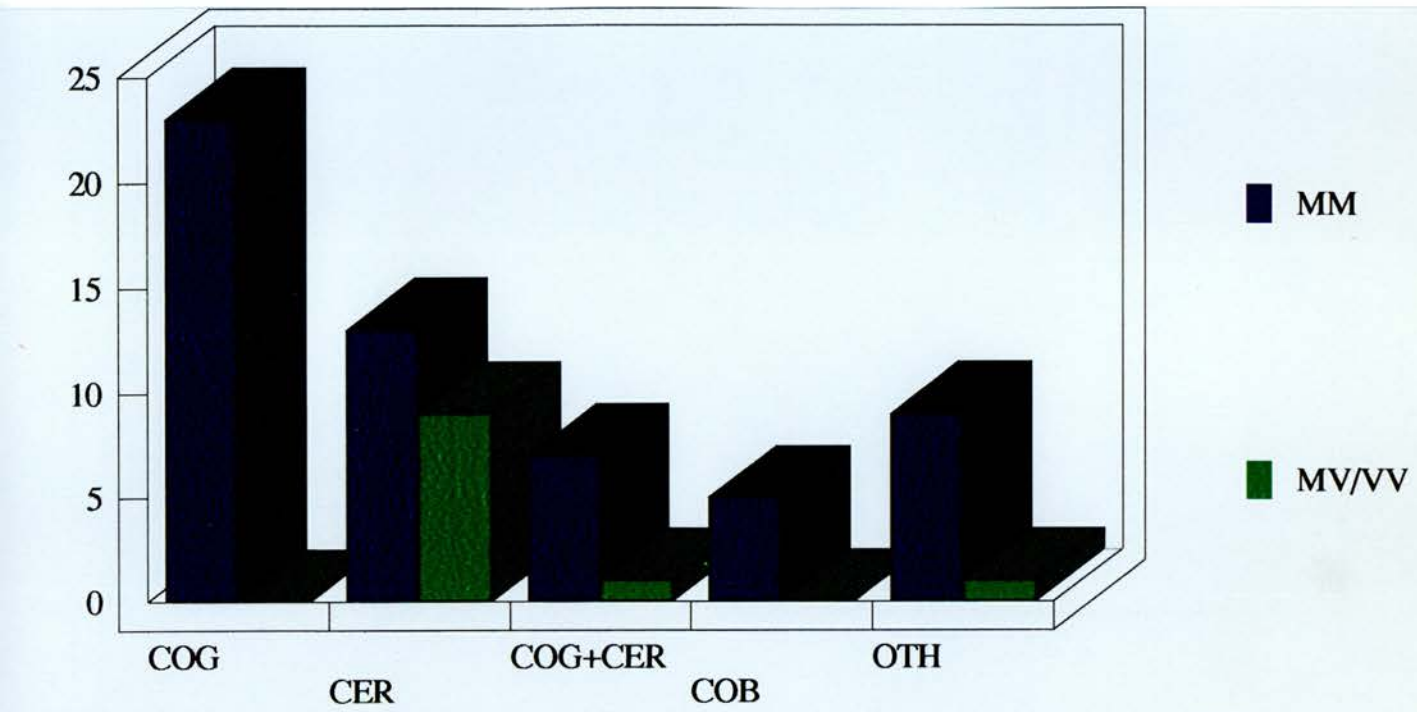
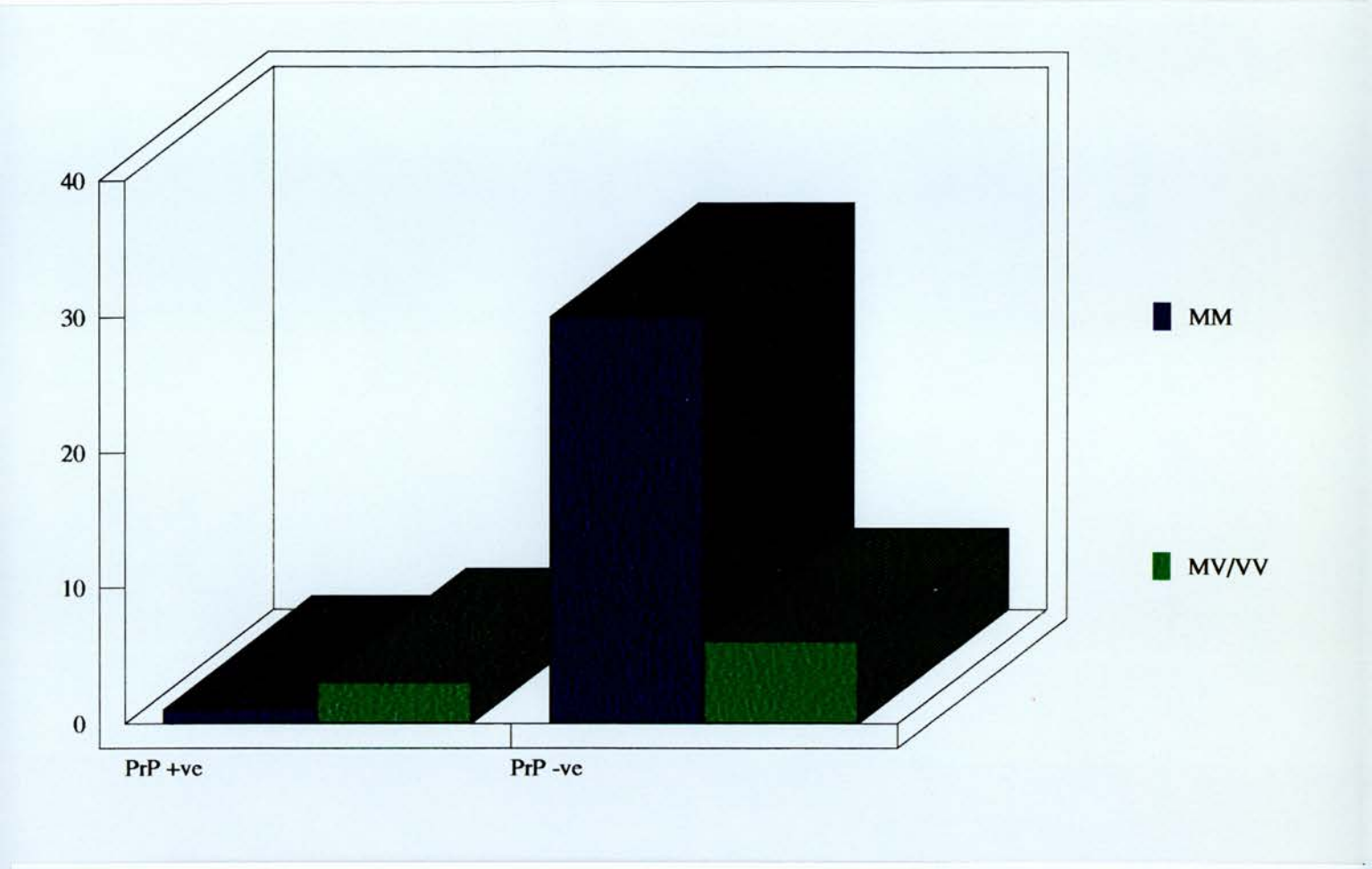


Figure 14: Comparison of presenting clinical features



Legend for Figure 14: Comparison of the presenting clinical features between cases that were Met homozygous at codon 129 (blue columns), and cases bearing Val alleles (green columns) at this site
Cerebellar dysfunction as a presenting feature is commoner in cases that are heterozygous or Val homozygous.

Figure 15: Codon 129 genotype in PrP+ve and -ve cases



Legend for Figure 15: Polymorphic status at codon 129 in sporadic CJD cases exhibiting (PrP +ve) and not exhibiting (PrP -ve) unicentric plaques
Met homozygous cases are shown in blue; heterozygous and Val homozygous cases are shown in green.
Cases bearing Val alleles are more likely to manifest plaques.

REFERENCES

1. Will, R.G. 1992 BSE and the spongiform encephalopathies. In *Recent advances in clinical neurology* (ed. C. Kennard), pp. 115-127. Edinburgh: Churchill Livingstone.

2. Wells, G.A.H., Scott, A.C., Johnson, C.T. *et al.* 1987 A novel progressive spongiform encephalopathy in cattle. *Vet. Rec.* **121**(18), 419-420.

3. Gibbs Jr., C.J., Joy, A., Heffner, R. *et al.* 1985 Clinical and pathological features and laboratory confirmation of Creutzfeldt- Jakob disease in a recipient of pituitary-derived human growth hormone. *N. Engl. J. Med.* **313**(12), 734-738.

4. Prusiner, S.B. 1982 Novel proteinaceous infectious particles cause scrapie. *Science* **218**, 136-144.

5. Meyer, A., Leigh, D. & Bagg, C.E. 1954 A rare presenile dementia associated with cortical blindness (Heidenhain's syndrome). *Journal of Neurology, Neurosurgery, and Psychiatry* **17**, 129-133.

6. Brownell, B. & Oppenheimer, D.R. 1965 An ataxic form of subacute presenile polioencephalopathy (Creutzfeldt-Jakob disease). *Journal of Neurology, Neurosurgery, and Psychiatry* **28**, 350-361.

7. Medori, R., Tritschler, H.J., LeBlanc, A. *et al.* 1992 Fatal familial insomnia, a prion disease with a mutation at codon 178 of the prion protein gene. *N. Eng. J. Med.* **326**, 444-449.

8. Brown, P., Preece, M.A. & Will, R.G. 1992 "Friendly fire " in medicine: hormones, homografts, and Creutzfeldt-Jakob disease. *Lancet* **340**, 24-27.

9. Bell, J.E. & Ironside, J.W. 1993 Neuropathology of spongiform encephalopathies in humans. In *British medical bulletin* **49**(4) (ed. I.V. Allen), pp. 738-777. Edinburgh: Churchill Livingstone.

10. Hayward, P.A.R., Bell, J.E. & Ironside, J.W. 1994 Prion protein imunocytochemistry: reliable protocols for the investigation of Creutzfeldt-Jakob disease. *Neuropathology and applied neurobiology* **20**, 375-383.

11. Robakis, N.K., Devine Gage, E.A., Jenkins, E.C. *et al.* 1986 Localization of a human gene homologous to the PrP gene on the p arm of chromosome 20 and detection of PrP-related antigens in normal human brain. *Biochem. biophys. Res. Commun.* **140**, 758-765.

12. Southwood committee 1989 *Report of the working party on Bovine Spongiform Encephalopathy.* Department of Health and Ministry of Agriculture, Fisheries and Food.

13. Creutzfeldt, H.G. 1920 Über eine eigenartige herdförmige Erkrankung des Zentralnervensystems. *Zeitschrift für die gesamte Neurologie und Psychiatrie* **57**, 1-18.

14. Jakob, A. 1921 Über eine der multiplen Sklerose klinisch nahestehende Erkrankung des Zentralnervensystems (spastische Pseudosklerose) mit bemerkenswertem anatomischem Befunde. Mitteilung eines vierten Falles. *Med. Klin.* **17**, 372-376.

15. Jakob, A. 1923 *Die Extrapyramidalen Erkrankungen*, pp. 218-245. Berlin: Springer.

16. Masters, C.L. & Gajdusek, D.C. 1982 The spectrum of Creutzfeldt-Jakob disease and the virus induced subacute spongiform encephalopathies. In *Recent advances in neuropathology* (ed. W. Thomas Smith and J.B. Cavanagh), pp. 139-144. Edinburgh: Churchill Livingstone.

17. Kirschbaum, W.R. 1968 *Jakob-Creutzfeldt Disease*, pp. 13-113. New York: Elsevier.

18. Will, R.G. 1984 The epidemiology of Creutzfeldt-Jakob disease. *MD thesis.* University of Cambridge.

19. Jones, D.P. & Nevin, S. 1954 Rapidly progressive cerebral degeneration (subacute vascular encephalopathy) with mental disorder, focal disturbances, and myolonic epilepsy. *Journal of Neurology, Neurosurgery, and Psychiatry* **17**, 148-159.

20. Nevin, S., McMenemey, W.H., Behrman, S. & Jones, D.P. 1960 Subacute spongiform encephalopathy- a subacute form of encephalopathy attributable to vascular dysfunction (spongiform cerebral atrophy). *Brain* **83**, 519-564.

21. Heidenhain, A. 1929 Klinische und Anatomische Untersuchungen über eine eigenartige Erkrankung des Zentralnervensystems im Praesenium. *Z. Ges. Neurol. Psychiat.* **118**, 49.

22. Gerstmann, J. 1928 Über ein noch nicht beschriebenes Reflexphänomen bei einer Erkrankung des zerebellaren Systems. *Weiner Medizinische Wochenschrift* **78**, 906-908.

23. Gerstmann, J., Sträussler, E. & Scheinker, I. 1936 Über eine eigenartige hereditär-familiäre Erkrankung des Zentralnervensystems. Zugleich ein Beitrag zur Frage des vorzeitigen lokalen Alterns. *Zeitschrift für Neurologie* **154**, 736-762.

24. Masters, C.L., Gajdusek, D.C. & Gibbs Jr., C.J. 1981 Creutzfeldt-Jakob disease virus isolations from the Gerstmann-Sträussler syndrome with an analysis of the various forms of amyloid plaque deposition in the virus-induced spongiform encephalopathies. *Brain* **104**, 559-588.

25. Hsiao, K., Baker, H.F., Crow, T.J. *et al.* 1989 Linkage of a prion protein missense variant to Gerstmann-Sträussler syndrome. *Nature* **338**, 342-345.

26. Kretzschmar, H.A., Honold, G., Seitelberger, F. *et al.* 1991 Prion protein mutation in family first reported by Gerstmann, Sträussler, and Scheinker. *Lancet* **337**, 1160.

27. Gajdusek, D.C. & Zigas, V. 1959 Kuru. Clinical, pathological and epidemiological study of an acute progressive degenerative disease of the central nervous system among natives of the Eastern Highlands of New Guinea. *Am. J. Med.* **26**, 442-469.

28. Simpson, D.A., Lander, H. & Robson, H.N. 1959 Observations on kuru: II. Clinical features. *Australas. ann. Med.* **8**, 8-15.

29. Hornabrook, R.W. 1979 Kuru and clinical neurology. In *Slow transmissible diseases of the nervous system* **1** (ed. S.B. Prusiner and W.J. Hadlow), pp. 37-66. New York: Academic Press.

30. Hadlow, W.J. 1959 Scrapie and kuru. *Lancet* **2**(7097), 289.

31. Gajdusek, D.C., Gibbs, C.J. & Alpers, M. 1966 Experimental transmission of a kuru-like syndrome to chimpanzees. *Nature* **209**, 794-796.

32. Alpers, M.A. 1979 Epidemiology and ecology of kuru. In *Slow transmissible diseases of the nervous system* **1** (ed. S.B. Prusiner and W.J. Hadlow), pp. 67-90.

33. Gibbs Jr., C.J., Gajdusek, D.C., Asher, D.M. *et al.* 1968 Creutzfeldt-Jakob disease (spongiform encephalopathy). Transmission to the chimpanzee. *Science* **161**, 388-389.

34. Galvez, S., Masters, C. & Gajdusek, D.C. 1980 Descriptive epidemiology of Creutzfeldt-Jakob disease in Chile. *Arch. Neurol.* **37**, 11-14.

35. Tsuji, S. & Kuroiwa, Y. 1983 Creutzfeldt-Jakob disease in Japan. *Neurology* **33**, 1503-1506.

36. Brown, P., Cathala, F., Raubertas, R.F. *et al.* 1987 The epidemiology of Creutzfeldt-Jakob disease. Conclusions of a 15 year investigation in France and review of the world literature. *Neurology* **37**, 895-904.

37. Harries-Jones, R., Knight, R., Will, R.G. *et al.* 1988 Creutzfeldt-Jakob disease in England and Wales, 1980-84: A case-control study of potential risk factors. *Journal of Neurology, Neurosurgery, and Psychiatry* **51**, 1113-1119.

38. Masters, C.L., Harris, J.O., Gajdusek, C. *et al.* 1978 Creutzfeldt- Jakob disease: Patterns of worldwide occurrence and the significance of familial and sporadic clustering. *Annals of Neurology* **5**(2), 177-188.

39. Brown, P. & Cathala, F. 1978 Creutzfeldt-Jakob disease in France: I. Retrospective study of the Paris area during the ten-year period 1968-1977. *Annals of Neurology* **5**(2), 189-192.

40. Brown, P., Cathala, F., Castaigne, P. *et al.* 1986 Creutzfeldt-Jakob disease: Clinical analysis of a consecutive series of 230 neuropathologically verified cases. *Annals of Neurology* **20**(5), 597-602.

41. Brown, P., Gibbs Jr., C.J., Rodgers-Johnson, P. *et al.* 1994 Human spongiform encephalopathy: the National Institutes of Health series of 300 cases of experimentally transmitted disease. *Annals of Neurology* **35**(5), 513-529.

42. Will, R.G. & Matthews, W.B. 1984 A retrospective study of Creutzfeldt-Jakob disease in England and Wales 1970-79 I: Clinical features. *Journal of Neurology, Neurosurgery, and Psychiatry* **47**, 134-140.

43. Masters, C.L., Gajdusek, D.C. & Gibbs, C.J. 1981 The familial occurence of Creutzfeldt-Jakob disease and Alzheimer's disease. *Brain* **104**, 535-558.

44. Will, R.G., Matthews, W.B., Smith, P.G. *et al.* 1986 A retrospective study of Creutzfeldt-Jakob disease in England and Wales 1970-1979 II: Epidemiolgy. *Journal of Neurology, Neurosurgery, and Psychiatry* **49**, 749-755.

45. Goldfarb, L.G., Brown, P. & Gajdusek, D.C. 1992 The molecular genetics of human transmissible spongiform encephalopathy. In *Prion diseases of humans and animals* (ed. S. Prusiner, J. Collinge, J. Powell and B. Anderton), pp. 139-153. London: Ellis Horwood.

46. Cathala, F. & Baron, H. 1987 Clinical aspects of Creutzfeldt-Jakob disease. In *Prions Novel infectious pathogens causing scrapie and Creutzfeldt-Jakob disease* (ed. S.B. Prusiner and M.P. McKinley), pp. 467-509. San Diego: Academic Press, Inc.

47. Knight, R. 1987 Transmissible dementia: clinical aspects. In *Degenerative neurological disease in the elderly* (ed. R.A. Griffiths and S.T. McCarthy), pp. 109-118. Bristol: Wright.

48. de Silva, R. & Esmonde, T. 1994 Iatrogenic transmission of Creutzfeldt-Jakob disease. *CNS Drugs* **2**(2), 96-101.

49. Advisory Committee on Dangerous Pathogens. 1994 Precautions for work with human and animal transmissible spongiform encephalopathies. London: HMSO.

50. Harrington, M.G., Merril, C.R., Asher, D.M. *et al.* 1986 Abnormal proteins in the cerebrospinal fluid of patients with Creutzfeldt-Jakob disease. *N Engl J Med* **315**, 279-283.

51. Blisard, K.S., Davis, L.E., Harrington, M.G. *et al.* 1990 Pre-mortem diagnosis of Creutzfeldt-Jakob disease by detection of abnormal cerebrospinal fluid proteins. *J Neurol Sci* **99**, 75-81.

52. Hsich, G., Kenney, K., Gibbs Jr., C.J. *et al.* 1996 The 14-3-3 brain protein in cerebrospinal fluid as a marker for transmissible spongiform encephalopathies. *N Engl J Med* **335**, 924-930.

53. Brown, P., Rodgers-Johnson, P., Cathala, F. *et al.* 1984 Creutzfeldt-Jakob disease of long duration: clinicopathological characteristics, transmissibility, and differential diagnosis. *Annals of Neurology* **16**(3), 295-304.

54. Salazar, A.M., Masters, C.L., Gajdusek, D.C. *et al.* 1983 Syndromes of amyotrophic lateral sclerosis and dementia: relation to transmissible Creutzfeldt-Jakob disease. *Annals of Neurology* **14**(1), 17-26.

55. Neary, D. 1990 Non Alzheimer's disease forms of cerebral atrophy. *Journal of Neurology, Neurosurgery, and Psychiatry* **53**, 929-931.

56. Udaka, F., Sawada, H., Seriu, N. *et al.* 1992 MRI and SPECT findings in amyotrophic lateral sclerosis. Demonstration of upper motor neurone involvement by clinical neuroimaging. *Neuroradiology* **34**(5), 389-393.

57. Kitagawa, Y., Gotoh, F., Koto, A. *et al.* 1983 Creutzfeldt-Jakob disease: a case with extensive white matter degeneration and optic atrophy. *J Neurol* **229**(2), 97-101.

58. Macchi, G., Abbamondi, A.L., Di Trapani, G. *et al.* 1984 On the white matter lesions of Creutzfeldt-Jakob disease. Can a new subentity be recognised in man? *J Neurol Sci* **63**(2), 197-206.

59. Kawata, A., Suga, M., Oda, M. *et al.* 1992 Creutzfeldt-Jakob disease with congophilic kuru plaques: CT and pathological findings of the cerebral white matter. *Journal of Neurology, Neurosurgery, and Psychiatry* **55**, 849-851.

60. Doh-ura, K., Kitamoto, T., Sakaki, Y. *et al.* 1991 CJD discrepancy. *Nature* **353**, 801-802.

61. Bolton, D.C., McKinley, M.P. & Prusiner, S.B. 1982 Identification of a protein that purifies with the scrapie prion. *Science* **218**, 1309-1311.

62. Prusiner, S.B., Bolton, D.C., Groth, D.F. *et al.* 1982 Further purification and characterization of scrapie prions. *Biochemistry* **21**, 6942-6950.

63. Bockman, J.M. 1991 Physicochemical and biological properties of the transmissible agent. In *Creutzfeldt-Jakob disease and other transmissible spongiform encephalopathies* (ed. F.O. Bastion), pp. 11-35.

64. Hsiao, K.K., Scott, M., Foster, D. *et al.* 1990 Spontaneous neurodegeneration in transgenic mice with mutant prion protein. *Science* **250**, 1587-1590.

65. Hsiao, K.K., Groth, D., Scott, M. *et al.* 1992 Genetic and transgenic studies of prion proteins in Gerstmann-Sträussler-Scheinker disease. In *Prion diseases of humans and animals* (ed. S. Prusiner, J. Collinge, J. Powell and B. Anderton), pp. 120-128. London: Ellis Horwood.

66. Prusiner, S.B. 1994 Molecular biology and genetics of prion diseases. *Phil. Trans. R. Soc. Lond. B* **343**, 447-463.

67. Büeler, H., Aguzzi, A., Sailer, A. *et al.* 1993 Mice devoid of PrP are resistant to scrapie. *Cell* **73**, 1339-1347.

68. Kocisko, D.A., Come, J.H., Priola, S.A. *et al.* 1994 Cell-free formation of protease-resistant prion protein. *Nature* **370**, 471-474.

69. Bruce, M.E. 1993 Scrapie strain variation and mutation. In *British medical bulletin* **49**(4) (ed. I.V. Allen), pp. 822-838. Edinburgh: Churchill Livingstone.

70. Kimberlin, R.H. 1990 Transmissible encephalopathies in animals. *Can J Vet Res* **54**, 30-37.

71. Weissmann, C. 1991 A "unified theory" of prion propagation. *Nature* **352**, 679-683.

72. Caughey, B. 1994 Scrapie-associated PrP accumulation and agent replication: effects of sulphated glycosaminoglycan analogues. *Phil. Trans. R. Soc. Lond. B* **343**, 399-404.

73. Baldwin, M.A., Pan, K-M., Nguyen, J. *et al.* 1994 Spectroscopic characterization of conformational differences between PrP^{C} and PrP^{Sc}: an α-helix to β-sheet transition. *Phil. Trans. R. Soc. Lond. B* **343**, 435-441.

74. Kitamoto, T., Amano, N., Terao, Y. *et al.* 1993 A new inherited prion disease (PrP-P105L mutation) showing spastic paraparesis. *Annals of Neurology* **34**(6), 808-813.

75. Doh-ura, K., Tateishi, J., Sasaki, H. *et al.* 1989 Pro-leu change at position 102 of prion protein is the most common but not the sole mutation related to Gerstmann-Sträussler syndrome. *Biochem. biophys. Res. Commun.* **163**, 974-979.

76. Hsiao, K.K., Cass, C., Schellenberg, G.D. *et al.* 1991 A prion protein variant in a family with the telencephalic form of Gerstmann-Sträussler-Scheinker syndrome. *Neurology* **41**, 681-684.

77. Kitamoto, T., Iizuka, R. & Tateishi, J. 1993 An amber mutation of prion protein in Gerstmann-Sträussler syndrome with mutant PrP plaques. *Biochem. Biophys. Res. Commun.* **192**, 525-531.

78. Goldfarb, L.G., Haltia, M., Brown, P. *et al.* 1991 New mutation in scrapie amyloid precursor gene (at codon 178) in Finnish Creutzfeldt-Jakob kindred. *Lancet* **337**, 425.

79. Kitamoto, T., Ohta, M., Doh-ura, K. *et al.* 1993 Novel missense variants of prion protein in Creutzfeldt-Jakob disease or Gerstmann-Sträussler syndrome. *Biochemical and biophysical research communications* **191**(2), 709-714.

80. Dlouhy, S.R., Hsiao, K., Farlow, M.R. *et al.* 1992 Linkage of the Indiana kindred of Gerstmann-Sträussler-Scheinker disease to the prion protein gene. *Nature Genetics* **1**, 64-67.

81. Goldgaber, D., Goldfarb, L.G., Brown, P. *et al.* 1989 Mutations in familial Creutzfeldt-Jakob disease and Gerstmann-Sträussler-Scheinker syndrome. *Experimental neurology* **106**, 204-206.

82. Mastrianni, J.A., Iannicola, C., Myers, R. *et al.* 1995 Identification of a new mutation of the prion protein gene at codon 208 in a patient with Creutzfeldt-Jakob disease. *Neurology* **45 (Suppl 4)**, A201.

83. Pocchiari, M., Salvatore, M., Cutruzzolá, F. *et al.* 1993 A new point mutation of the prion protein gene in Creutzfeldt-Jakob disease. *Annals of Neurology* **34**(6), 802-807.

84. Hsiao, K., Dlouhy, S.R., Farlow, M.R. *et al.* 1992 Mutant prion protein in Gerstmann-Sträussler-Scheinker disease with neurofibrillary tangles. *Nature Genetics* **1**, 68-71.

85. Goldfarb, L.G., Brown, P., Little, B.W. *et al.* 1993 A new (two-repeat) octapeptide coding insert mutation in Creutzfeldt-Jakob disease. *Neurology* **43**, 2392-2394.

86. Campbell, T.A., Palmer, M.S., Will, R.G. *et al.* 1996 A prion disease with a novel 96-base pair insertional mutation in the prion protein gene. *Neurology* **46**, 761-766.

87. Goldfarb, L.G., Brown, P., McCombie, W.R. *et al.* 1991 Transmissible familial Creutzfeldt-Jakob disease associated with five, seven, and eight extra octapeptide coding repeats in the PRNP gene. *Proc. Natl. Acad. Sci. USA* **88**, 10926-10930.

88. Owen, F., Poulter, M., Shah, T. *et al.* 1990 An in-frame insertion in the prion protein gene in familial Creutzfeldt-Jakob disease. *Molecular Brain Research* **7**, 273-276.

89. Collinge, J., Brown, J., Hardy, J. *et al.* 1992 Inherited prion disease with 144 base pair gene insertion 2. Clinical and pathological features. *Brain* **115**, 687-710.

90. Nicholl, D., Windl, O., de Silva, R. *et al.* 1995 Inherited Creutzfeldt-Jakob disease in a British family associated with a novel 144 base pair insertion of the prion protein gene. *Journal of Neurology, Neurosurgery, and Psychiatry* **58**, 65-69.

91. Oda, T., Kitamoto, T., Tateishi, J. *et al.* 1995 Prion disease with 144 base pair insertion in a Japanese family line. *Acta Neuropathol* **90**, 80-86.

92. van Gool, W.A., Hensels, G.W., Hoogerwaard, E.M. *et al.* 1995 Hypokinesia and presenile dementia in a Dutch family with a novel insertion in the prion protein gene. *Brain* **118**, 1565-1571.

93. Owen, F., Poulter, M., Collinge, J. *et al.* 1992 A dementing illness associated with a novel insertion in the prion protein gene. *Molecular Brain Research* **13**, 155-157.

94. Duchen, L.W., Poulter, M. & Harding, A.E. 1993 Dementia associated with a 216 base pair insertion in the prion protein gene. *Brain* **116**, 555-567.

95. Poulter, M., Baker, H.F., Frith C.D. *et al.* 1992 Inherited prion disease with 144 base pair gene insertion 1. Genealogical and molecular studies. *Brain* **115**, 675-685.

96. Westaway, D., DeArmond, S.J., Cayetano-Canlas, J. *et al.* 1994 Degeneration of skeletal muscle, peripheral nerves and the CNS in mice overexpressing wild-type prion proteins. *Cell* **76**, 117-129.

97. Telling, G.C., Scott, M., Mastrianni, J. *et al.* 1995 Prion propagation in mice expressing human and chimeric PrP transgenes implicates the interaction of cellular PrP with another protein. *Cell* **83**(1), 79-90.

98. Collinge, J., Palmer, M.S. & Dryden, A.J. 1991 Genetic predisposition to iatrogenic Creutzfeldt-Jakob disease. *Lancet* **337**, 1441-1442.

99. Palmer, M.S., Dryden, A., Hughes, J.T. *et al.* 1991 Homozygous prion protein genotype predisposes to sporadic Creutzfeldt-Jakob disease. *Nature* **352**, 340-342.

100. Brown, P., Cervenáková, L., Goldfarb, L.G. *et al.* 1994 Iatrogenic Creutzfeldt-Jakob disease: an example of the interplay between ancient genes and modern medicine. *Neurology* **44**, 291-293.

101. Laplanche, J.-L., Delasnerie-Lauprêtre, N., Brandel, J.P. *et al.* 1994 Molecular genetics of prion diseases in France. *Neurology* **44**, 2347-2351.

102. Windl, O., Dempster, M., Estibeiro, J.P. *et* al. 1996 Genetic basis of Creutzfeldt-Jakob disease in the United Kingdom: a systematic analysis of predisposing mutations and allelic variation in the PRNP gene. *Hum* Genet **98**, 259-264.

103. Brown, P. 1992 The phenotypic expression of different mutations in transmissible human spongiform encephalopathy. *Rev. Neurol. (Paris)* **148**(5), 317-327.

104. Chapman, J., Brown, P., Goldfarb, L.G. *et al.* 1993 Clinical heterogeneity and unusual presentations of Creutzfeldt-Jakob disease in Jewish patients with the PRNP codon 200 mutation. *Journal of Neurology, Neurosurgery, and Psychiatry* **56**, 1109-1112.

105. Mastrianni, J.A., Curtis, M.T., Oberholtzer, J.C. *et al.* 1994 Ataxic Gerstmann-Sträussler-Scheinker disease caused by a prion protein gene mutation at codon 117. *Neurology* **44** (4, Suppl 2), A260.

106. Goldfarb, L.G., Petersen, R.B., Tabaton, M. *et al.* 1992 Fatal familial insomnia and familial Creutzfeldt-Jakob disease: disease phenotype determined by a DNA polymorphism. *Science* **258**, 806-808.

107. Barbanti, P., Fabbrini, G., Salvatore, M. *et al.* 1996 Polymorphism at codon 129 or codon 219 of PRNP and clinical heterogeneity in a previously unreported family with Gerstmann-Sträussler-Scheinker disease (PrP-P102L mutation). *Neurology* **47**, 734-741.

108. Collinge, J. & Palmer, M.S. 1994 Molecular genetics of human prion diseases. *Phil. Trans. R. Soc. Lond. B* **343**, 371-378.

109. Brown, P., Goldfarb, L.G., Brown, W.T. *et al.* 1991 Clinical and molecular genetic study of a large German kindred with Gerstmann-Sträussler-Scheinker syndrome. *Neurology* **41**, 375-379.

110. Nochlin, D., Sumi, S.M., Bird, T.D. *et al.* 1989 Familial dementia with PrP-positive amyloid plaques: a variant of Gerstmann-Stäussler syndrome. *Neurology* **39**, 910-918.

111. Ghetti, B., Tagliavini, F., Hsiao, K. *et al.* 1992 Indiana variant of Gerstmann-Sträussler-Scheinker disease. In *Prion diseases of humans and animals* (ed. S. Prusiner, J. Collinge, J. Powell and B. Anderton), pp. 154-167. London: Ellis Horwood.

112. Miyazono, M., Kitamoto, T., Doh-ura, K. *et al.* 1992 Creutzfeldt-Jakob disease with codon 129 polymorphism (Valine): a comparative study of patients with codon 102 point mutation or without mutations. *Acta Neuropathologica* **84**, 349-354.

113. de Silva, R., Ironside, J.W., McCardle, L. *et al.* 1994 Neuropathological phenotype and "prion protein" genotype correlation in sporadic Creutzfeldt-Jakob disease. *Neuroscience Letters* **179**, 50-52.

114. Pickering-Brown, S.M., Mann, D.M.A., Owen, F. *et al.* 1995 Allelic variations in apolipoprotein E and prion protein genotype related to plaque formation and age of onset in sporadic Creutzfeldt-Jakob disease. *Neuroscience Letters* **187**, 127-129.

115. de Silva, R., Ironside, J.W., Barrie, C. *et al.* 1994 Amyloid plaques in Creutzfeldt-Jakob disease: prevalence and clinical correlates. *Annals of Neurology* **36**(2), 273.

116. Herzberg, L., Herzberg, B.N., Gibbs, C.J.Jr. *et al.* 1974 Creutzfeldt-Jakob disease: hypothesis for high incidence in Libyan jews in Israel. *Science* **13**, 843.

117. Hsiao, K., Meiner, Z., Kahana, E. *et al.* 1991 Mutation of the prion protein in Libyan jews with Creutzfeldt-Jakob disease. *New England Journal of Medicine* **324**(16), 1091-1097.

118. Goldfarb, L.G., Mitrová, E., Brown, P. *et al.* 1990 Mutation in codon 200 of scrapie amyloid protein gene in two clusters of Creutzfeldt-Jakob disease in Slovakia. *Lancet* **336**, 514-515.

119. Brown, P., Galvez, S., Goldfarb, L.G. *et al.* 1992 Familial Creutzfeldt-Jakob disease in Chile is associated with the codon 200 mutation of the PRNP amyloid precursor gene on chromosome 20. *J. Neurol. Sci.* **112**, 65-67.

120. Collinge, J., Palmer, M.S., Campbell, T.A. *et al.* 1993 Inherited prion disease (PrP lysine 200) in Britain: two case reports. *Br. Med. J.* **306**, 301-302.

121. Laplanche, J.-L., Chatelain, J., Dussaucy, M. *et al.* 1993 Inherited prion disease. *Br. Med. J.* **306**, 794-795.

122. Inoue, I., Kitamoto, T., Doh-ura, K. *et al.* 1994 Japanese family with Creutzfeldt-Jakob disease with codon 200 point mutation of the prion protein gene. *Neurology* **44**, 299-301.

123. Neufeld, M.Y., Josiphov, J. & Korczyn, A.D. 1992 Demyelinating peripheral neuropathy in Creutzfeldt-Jakob disease. *Muscle & Nerve* **15**, 1234-1239.

124. Chapman, J., Arlazoroff, A., Goldfarb, L.G. *et al.* 1996 Fatal insomnia in a case of familial Creutzfeldt-Jakob disease with the codon 200^{Lys} mutation. *Neurology* **46**, 758-761.

125. Goldfarb, L.G., Brown, P., Mitrová, E. *et al.* 1991 Creutzfeldt-Jakob disease associated with the PRNP codon 200Lys mutation: an analysis of 45 families. *Eur. J. Epidemiol.* **7**, 477-486.

126. Chapman, J., Ben-Israel, J., Goldhammer, Y. & Korczyn, A.D. 1994 The risk of developing Creutzfeldt-Jakob disease in subjects with the PRNP gene codon 200 point mutation. *Neurology* **44**, 1683-1686.

127. Brown, P., Cervenáková, L., Boellaard, J.W *et al.* 1994 Identification of a PRNP gene mutation in Jakob's original Creutzfeldt-Jakob disease family. *Lancet* **344**, 130-131.

128. Kirschbaum, W.R. 1924 Zwei eigenartige Erkrankungen des Zentralnervensystems nach Art der spastischen Pseudosclerose (Jakob). *Z. Ges. Neurol. Psychiat.* **92**, 175-202.

129. Lugaresi, E., Medori, R., Montagna, P. *et al.* 1986 Fatal familial insomnia and dysautonomia with selective degeneration of thalamic nuclei. *New England Journal of Medicine* **313**(16), 997-1003.

130. Prusiner, S.B. 1994 Inherited prion diseases. *Proc. Natl. Acad. Sci. USA* **91**, 4611-4614.

131. Tateishi, J., Brown, P., Kitamoto, T. *et al.* 1995 First experimental transmission of fatal familial insomnia. *Nature* **376**, 434-435.

132. Collinge, J., Palmer, M.S., Sidle, K.C.L. *et al.* 1995 Transmission of fatal familial insomnia to laboratory animals. *Lancet* **346**, 569-570.

133. Owen, F., Poulter, M., Collinge, J. *et al.* 1991 Insertions in the prion protein gene in atypical dementias. *Experimental Neurology* **112**, 240-242.

134. Collinge, J., Owen, F., Poulter, M. *et al.* 1990 Prion dementia without characteristic pathology. *Lancet* **336**, 7-9.

135. Clinton, J., Lantos, P.L., Rossor, M. *et al.* 1990 Immunocytochemical confirmation of prion protein. *Lancet* **336**, 515.

136. La Spada, A.R., Paulson, H.L. & Fischbeck, K.H. 1994 Trinucleotide repeat expansion in neurological disease. *Ann Neurol* **36**, 814-821.

137. Goodbrand, I.A., Ironside, J.W., Nicolson, D. *et al.* 1995 Prion protein accumulation in the spinal cords of patients with sporadic and growth hormone associated Creutzfeldt-Jakob disease. *Neuroscience Letters* **183**, 127-130.

138. Kitamoto, T. & Tateishi, J. 1994 Human prion diseases with variant prion protein. *Phil. Trans. R. Soc. Lond. B* **343**, 391-398.

139. Poirier, J., Davignon, J., Bouthillier, D. *et al.* 1993 Apolipoprotein E polymorphism and Alzheimer's disease. *Lancet* **342**, 697-699.

140. Pickering-Brown, S.M., Mann, D.M.A., Bourke, J.P. *et al.* 1994 Apolipoprotein E4 and Alzheimer's disease pathology in Lewy body disease and in other beta-amyloid forming diseases. *Lancet* **343**, 1155.

141. Hardy, J., Crock, R., Perry, R. *et al.* 1994 Apo E genotype and Down's syndrome. *Lancet* **343**, 979-980.

142. Amouyel, P., Vidal, O., Launay, J.M. *et al.* 1994 The apolipoprotein E alleles as major susceptibility factors for Creutzfeldt-Jakob disease. *Lancet* **344**, 1315-1318.

143. Zerr, I., Helmhold, M. & Weber, T. 1994 Apolipoprotein E in Creutzfeldt-Jakob disease. *Lancet* **345**, 68-69.

144. Zerr, I., Helmhold, M., Poser, S. *et al.* 1996 Apolipoprotein E phenotype frequency and cerebrospinal fluid concentration are not associated with Creutzfeldt-Jakob disease. *Arch Neurol* **53**, 1233-1238.

145. Büeler, H., Fischer, M., Lang, Y. *et al.* 1992 Normal development and behaviour of mice lacking the neuronal cell-surface PrP protein. *Nature* **356**, 577-582.

146. Collinge, J., Whittington, M.A., Sidle, K.C.L. *et al.* 1994 Prion protein is necessary for normal synaptic function. *Nature* **370**, 295-297.

147. Tobler, I., Gaus, S.E., Deboer, T. *et al.* 1996 Altered circadian activity rhythms and sleep in mice devoid of prion protein. *Nature* **380**, 639-642.

148. Koch, T.K., Berg, B.O., De Armond, S.J. *et al.* 1985 Creutzfeldt-Jakob disease in a young adult with idiopathic hypopituitarism. Possible relation to the administration of cadaveric human growth hormone. *New England Journal of Medicine* **313**(12), 731-733.

149. Cochius, J.I., Hyman, N. & Esiri, M.M. 1992 Creutzfeldt-Jakob disease in a recipient of human pituitary-derived gonadotrophin: a second case. *Journal of Neurology, Neurosurgery, and Psychiatry* **55**, 1094-1095.

150. Healy, D.L. & Evans, J. 1993 Creutzfeldt-Jakob disease after pituitary gonadotrophins. *British Medical Journal* **307**, 517-518.

151. Brown, P. 1988 The clinical neurology and epidemiology of Creutzfeldt-Jakob disease, with special reference to iatrogenic cases. In *Novel infectious agents and the central nervous system* (ed. G. Bock and J. Marsh), pp. 3-23. Chichester: John Wiley & Sons.

152. Brown, P. 1996 Environmental causes of human spongiform encephalopathy. In *Prion* diseases (ed. H.F. Baker and R.M. Ridley), pp. 139-154. Totowa: Humana Press Inc.

153. Martínez-lage, J.F., Poza, M., Sola, J. *et al.* 1994 Accidental transmission of Creutzfeldt-Jakob disease by dural cadaveric grafts. *Journal of Neurology, Neurosurgery, and Psychiatry* **57**, 1091-1094.

154. Duffy, P., Wolf, J., Collins, G. *et al.* 1974 Possible person-to-person transmission of Creutzfeldt-Jakob disease. *N Engl J Med* **290**, 692.

155. Manuelidis, E.E., Angelo, J.N., Gorgacz, E.J. *et al.* 1977 Experimental Creutzfeldt-Jakob disease transmitted via the eye with infected cornea. *N Engl J Med* **296**, 1334-1336.

156. Liberski, P.P., Yanagihara, R., Gibbs, C.J.Jr. *et al.* 1990 Spread of Creutzfeldt-Jakob disease virus along visual pathways after intraocular inoculation. *Arch Virol* **111**, 141-147.

157. Bernoulli, C., Siegfried, J., Baumgartner, G. *et al.* 1977 Danger of accidental person-to-person transmission of Creutzfeldt-Jakob disease by surgery. *Lancet* **1**, 478-479.

158. Gibbs, C.J.Jr., Asher, D.M., Kobrine, A. *et al.* 1994 Transmission of Creutzfeldt-Jakob disease to a chimpanzee by electrodes contaminated during neurosurgery. *Journal of Neurology, Neurosurgery, and Psychiatry* **57**, 757-758.

159. Will, R.G. & Matthews, W.B. 1982 Evidence for case-to-case transmission of Creutzfeldt-Jakob disease. *Journal of Neurology, Neurosurgery, and Psychiatry* **45**, 235-238.

160. Foncin, J.F., Gaches, J., Cathala, F. *et al.* 1980 Transmission iatrogéne interhumaine possible de maladie de Creutzfeldt-Jakob avec atteinte des grains du cervelet. *Rev Neurol (Paris)* **136**(3), 280.

161. National Creutzfeldt-Jakob Disease Surveillance Unit. 1994 Creutzfeldt-Jakob disease surveillance in the United Kingdom. Third annual report.

162. Will, R.G., Ironside, J.W., Zeidler, M. *et al.* 1996 A new variant of Creutzfeldt-Jakob disease in the UK. *Lancet* **347**, 921-925.

163. Chazot, G., Broussolle, E., Lapras, C.I. *et al.* 1996 New variant of Creutzfeldt-Jakob disease in a 26-year-old French man. *Lancet* **347**, 1181.

164. Will, R.G., Zeidler, M., Brown, P. *et al.* 1996 Cerebrospinal-fluid test for new-variant Creutzfeldt-Jakob disease. *Lancet* **348**, 955.

165. Collinge, J., Sidle, K.C.L., Meads, J. *et al.* 1996 Molecular analysis of prion strain variation and the aetiology of "new variant" CJD. *Nature* **383**, 685-690.

166. Hill, A.F., Zeidler, M., Ironside, J. & Collinge, J. 1997 Diagnosis of new variant Creutzfeldt-Jakob disease by tonsil biopsy. *Lancet* **349**, 99-100.

167. McNaughton, H.K. & Will, R.G. 1997 Creutzfeldt-Jakob disease presenting acutely as stroke; an analysis of 30 cases. *Neurological Infections and Epidemiology* **2**, 19-24.

168. Muramoto, T., Kitamoto, T., Koga, H. *et al.* 1992 The coexistance of Alzheimer's disease and Creutzfeldt-Jakob disease in a patient with dementia of long duration. *Acta Neuropathologica* **84**(6), 686-689.

169. Esmonde, T.G. & Will, R.G. 1992 Creutzfeldt-Jakob disease in Scotland and Northern Ireland. *Scot Med J* **37**, 181-184.

170. Biomed I programme for the surveillance of Creutzfeldt-Jakob disease in the European Community. 1994 Yearly activity report.

171. Harper, P.S. 1993 Research samples from families with genetic diseases: a proposed code of conduct. *Br Med J* **306**, 1391-1394.

172. National Creutzfeldt-Jakob Disease Surveillance Unit. 1993 Creutzfeldt-Jakob disease surveillance in the United Kingdom. Second annual report.

173. National CJD Surveillance Unit. 1995 Creutzfeldt-Jakob disease surveillance in the United Kingdom. Fourth annual report.

174. Orita, M., Iwahana, H., Kanazawa, H. *et al.* 1989 Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. *Proc Natl Acad Sci USA* **86**, 2766-2770.

175. Estibeiro, J.P. 1987 Factors involved in 3' splice site selection in eukaryotic pre-mRNA. Thesis for the degree of PhD, University of Leicester.

176. Kretzschmar, H.A., Stowring, L.E., Westaway, D. *et al.* 1986 Molecular cloning of a human prion protein cDNA. *DNA* **5**, 315-324.

177. Tobias, E., Mann, C., Bone, I. *et al.* 1994 A case of Creutzfeldt- Jakob disease presenting with cortical deafness. *Journal of Neurology, Neurosurgery, and Psychiatry* **57**, 872-873.

178. Tanaka, M., Iizuka, O. & Yuasa, T. 1992 Hepatic dysfunction in Creutzfeldt-Jakob disease. *Neurology* **42**, 1249.

179. Tanaka, M. 1993 Liver in Creutzfeldt-Jakob disease. *Neurology* **43**, 457.

180. Uchino, A., Yoshinaga, M., Shiokawa, O. *et al.* 1991 Serial MR imaging in Creutzfeldt-Jakob disease. *Neuroradiology* **33**, 364-367.

181. Milton, W.J., Atlas, S.W., Lavi, E. *et al.* 1991 Magnetic resonance imaging of Creutzfeldt-Jakob disease. *Ann Neurol* **29**, 438-440.

182. Onofrj, M., Fulgente, T., Gambi, D. *et al.* 1993 Early MRI findings in Creutzfeldt-Jakob disease. *J Neurol* **240**, 423-426.

183. Esmonde, T.F.G. & Will, R.G. 1992 Magnetic resonance imaging in Creutzfeldt-Jakob disease. *Ann Neurol* **31**, 231.

184. Barker, D.J.P. & Rose, G. 1990 *Epidemiology in medical Practice.* Edinburgh: Churchill Livingstone.

185. Powers, J.M., Liu, Y., Hair, L.S. *et al.* 1991 Concomitant Creutzfeldt-Jakob and Alzheimer diseases. *Acta Neuropathologica* **83**(1), 95-98.

186. Almond, J.W., Brown, P., Gore, S.M. *et al.* 1995 Creutzfeldt-Jakob disease and bovine spongiform encephalopathy: any connection? *Br Med J* **311**, 1415-1421.

187. Sackett, D.L., Haynes, R.B., Guyatt, G.H. *et al.* 1991 The Clinical Examination. In *Clinical Epidemiology: A Basic Science for Clinical Medicine*, pp. 19-49. Boston: Little, Brown and Company.

188. Jellinger, K., Danielczyk, W., Fischer, P. *et al.* 1990 Clinicopathological analysis of dementia disorders in the elderly. *Journal of Neurological Sciences* **95**, 239-258.

189. Brown, P., Kaur, P., Sulima, M.P. *et al.* 1993 Real and imagined clinicopathological limits of "prion dementia". *Lancet* **341**, 127-129.

190. Hainfellner, J.A., Jellinger, K., Diringer, H. *et al.* 1996 Creutzfeldt-Jakob disease in Austria. *Journal of Neurology, Neurosurgery, and Psychiatry* **61**, 139-142.

191. Mastrianni, J.A., Curtis, M.T., Oberholtzer, J.C. *et al.* 1995 Prion disease (PrP-A117V) presenting with ataxia instead of dementia. *Neurology* **45**, 2042-2050.

192. Marsh, R.F., Sipe, J.C., Morse, S.S. *et al.* 1976 Transmissible mink encephalopathy: reduced spongiform degeneration in aged mink of the Chediak-Higashi genotype. *Lab Invest* **34**, 381-386.

193. Carlson, G.A., Ebeling, C., Yang, S-L *et al.* 1994 Prion isolate specified allotypic interactions between the cellular and scrapie prion proteins in congenic and transgenic mice. *Proc Natl Acad Sci USA* **91**, 5690-5694.

194. Petersen, R.B., Tabaton, M., Chen, S.G. *et al.* 1995 Familial progressive subcortical gliosis: presence of prions and linkage to chromosome 17. *Neurology* **45**, 1062-1067.

195. Brown, P., Kenney, K., Little, B. *et al.* 1995 Intracerebral distribution of infectious amyloid protein in spongiform encephalopathy. A*nn Neurol* **38**, 245-253.

196. Zerr, I., Bodemer, M., Otto, M. *et al.* 1996 Diagnosis of Creutzfeldt-Jakob disease by two-dimensional gel electrophoresis of cerebrospinal fluid. *Lancet* **348**, 846-849.

197. Zerr, I., Bodemer, M., Räcker, S. *et al.* 1995 Cerebrospinal fluid concentration of neuron-specific enolase in diagnosis of Creutzfeldt-Jakob disease. *Lancet* **345**, 1609-1610.

198. Yoon, S.S., Chan, S., Chin, S. *et al.* 1995 MRI of Creutzfeldt-Jakob disease: asymmetric high signal intensity of the basal ganglia. *Neurology* **45**, 1932-1933.

199. Zeidler, M., Will, R.G., Ironside, J.W. *et al.* 1996 Creutzfeldt-Jakob disease and bovine spongiform encephalopathy. Magnetic resonance imaging is not a sensitive test for Creutzfeldt-Jakob disease. *Br Med J* **312**(7034), 844.

200. Salvatore, M., Genuardi, M., Petraroli, R. *et al.* 1994 Polymorphisms of the prion protein gene in Italian patients with Creutzfeldt-Jakob disease. *Human Genetics* **94(4)**, 375-379.

201. Collinge, J., Owen, F., Lofthouse, R. *et al.* 1989 Diagnosis of Gerstmann-Sträussler syndrome in familial dementia with prion protein gene analysis. *Lancet* **July 1**, 15-17.

202. Collinge, J., Poulter, M., Davis, M.B. *et al.* 1991 Presymptomatic detection or exclusion of prion protein gene defects in families with inherited prion diseases. *Am J Hum Genet* **49**, 1351-1354.

203. Brown, P., Cervenáková, L., Goldfarb, L.G. *et al.* 1994 Molecular genetic testing of a fetus at risk of Gerstmann-Sträussler-Scheinker syndrome. *Lancet* **343**, 181-182.

204. Parchi, P., Castellani, R., Capellari, S. *et al.* 1996 Molecular basis of phenotypic variability in sporadic Creutzfeldt-Jakob disease. *Ann Neurol* **39**, 767-778.

205. Will, R.G. 1996 Surveillance of prion diseases in humans. In *Prion diseases* (ed. H.F. Baker and R.M. Ridley), pp. 119-137. Totowa: Humana Press Inc.

206. Skegg, D.C.G. 1997 Epidemic or false alarm? *Nature* **385**, 200.

207. Lasmézas, C.I., Deslys, J.P., Demaimay, R. *et al.* 1996 BSE transmission to macaques. *Nature* **381**, 743-744.

208. Aguzzi, A. & Weissmann, C. 1996 A suspicious signature. *Nature* **383**, 666-667.

209. Crompton, M.R. 1968 Alpers' disease- a variant of Creutzfeldt-Jakob disease and subacute spongiform encephalopathy? *Acta Neuropathologica* **10**(2), 99-104.

210. Manuelidis, E.E. & Rorke, L.B. 1989 Transmission of Alpers' disease (chronic progressive encephalopathy) produces experimental Creutzfeldt-Jakob disease in hamsters. *Neurology* **39**(5), 615-621.

211. Sakaguchi, S., Katamine, S., Nishida, N. *et al.* 1996 Loss of cerebellar Purkinje cells in aged mice homozygous for a disrupted *PrP* gene. *Nature* **380**, 528-531.

212. Alper, T., Cramp, W.A., Haig, D.A. & Clarke, M.C. 1967 Does the agent of scrapie replicate without nucleic acid? *Nature* **214**, 764-766.

APPENDICES

Legend for Appendix 1: Tabulation of cases seen by the various Neurologists in this study

Appendix 1

Neurologist	Cases examined
RGW	1,2,3,24,64,102,113,132,152,158,163,213, 214, 215, 234, 279,308,333 (n = 18)
TFGE	5,7,9,10,13,14,19,20,21,22,25,26,27,29,31,33,34, 35,39,42,43,44,45,46,47,50,51,53,54,55,56,57, 67,68,70,71,72,73,74,75,76,77,79,81,82,83,84, 85,86,89,90,97,101,105,109,110,111,112,114, 115,116,118,120,121,122,125,126,127,129,130, 131,136,139,143,144,145,146,148,149,151,155, 156,159,160 (n = 84)
MZ	205,251,299,316,339 (n = 5)
RDS	All other cases (n = 104)

N.B. Cases designated other# (see Methods), were seen by RGW, TFGE or RDS

[4,17,18,28,30,40,48,49,66,69,92,96,123,134,137,142,167,171,192,206,226, 238,241,258,263,266,270,296,315,330]

(n = 30).

Appendix 2: Data extracted from clinical record

First manifesting sign(s)*		Early features**	During course
Cognitive-	Personality Behaviour Memory Disorientation		
Dysphasia-	Expressive Receptive		
Motor dyspraxia			
Pyramidal dysfunction			
Extra-pyramidal dysfunction-	Parkinsonism Chorea Dystonia		
Myoclonus			
Epilepsia partialis continua			
Visual-	Agnosia/blindness Hallucinations		
Cerebellar-	Incoordination Nystagmus		
Oculomotor disturbance		As * , plus	
		Bulbar dysfunction	
		Muscle wasting	
		Incontinence	As ** , plus
			Primitive reflexes
			Facial weakness
			Paratonic rigidity
			Seizures
			Akinetic mutism
			Respiratory depression

Appendix 3: Patients whose molecular biological examinations were performed at St. Mary's Hospital.

1, 2, 9,13, 20, 21, 27, 43, 45, 46, 47, 50, 56, 57, 68, 234, 291, 301, 309, 19, 34, 39, 53, 55, 33, 51

(n = 26)

Legend for Appendix 4:

Frequency of clinical features in sporadic, familial and iatrogenic (via "peripheral" inoculation) CJD, at various stages of illness

Columns 1 - 3: at presentation

Columns 4 - 6: early in course

Columns 7 - 9: during illness

Appendix 4: Frequency of clinical features in sporadic, familial and (peripherally inoculated) iatrogenic CJD

Feature	1. Sporadic	2. Familial	3. Iatrogen.	4. Sporadic	5. Familial	6. Iatrogen.	7. Sporadic	8. Familial	9. Iatrogen.
	At presentation			Early in course			During illness		
Personality	0.140	0.210	0.000	0.510	0.360	0.220	0.530	0.430	0.330
Behaviour	0.150	0.210	0.000	0.520	0.500	0.000	0.620	0.640	0.110
Memory	0.190	0.140	0.110	0.570	0.430	0.110	0.640	0.640	0.560
Disorient.	0.150	0.140	0.000	0.620	0.790	0.000	0.780	0.860	0.220
Exp. dysp.	0.040	0.070	0.000	0.270	0.430	0.000	0.380	0.640	0.000
Rec. dysp.	0.000	0.000	0.000	0.050	0.070	0.000	0.200	0.360	0.000
Dyspraxia	0.030	0.000	0.000	0.440	0.360	0.110	0.550	0.710	0.330
Pyramidal	0.030	0.000	0.000	0.200	0.070	0.000	0.620	0.360	0.220
Parkinson.	0.010	0.000	0.000	0.130	0.210	0.000	0.340	0.290	0.110
Chorea	0.010	0.000	0.000	0.040	0.000	0.110	0.130	0.140	0.110
Dystonia	0.000	0.000	0.000	0.060	0.000	0.000	0.160	0.140	0.000
Myoclonus	0.010	0.000	0.000	0.350	0.500	0.440	0.850	0.860	0.780
EPC	0.000	0.000	0.000	0.020	0.000	0.000	0.040	0.000	0.000
Blindness	0.090	0.070	0.000	0.350	0.210	0.000	0.520	0.360	0.000
Hallucinat.	0.010	0.000	0.000	0.210	0.290	0.000	0.320	0.290	0.000
Incoordin.	0.390	0.210	0.890	0.760	0.710	1.000	0.850	0.860	1.000
Nystagmus	0.000	0.000	0.000	0.100	0.000	0.330	0.200	0.140	0.560
Oculomot.	0.010	0.000	0.000	0.140	0.210	0.220	0.270	0.360	0.560
Bulbar dys.	-	-	-	0.020	0.070	0.000	0.080	0.140	0.110
Mus. wast.	-	-	-	0.010	0.000	0.110	0.170	0.140	0.110
Incontin.	-	-	-	0.160	0.290	0.220	0.390	0.430	0.440
Primit. refl.	-	-	-	-	-	-	0.580	0.710	0.330
Facial wk.	-	-	-	-	-	-	0.160	0.070	0.110
PT rigidity	-	-	-	-	-	-	0.380	0.500	0.000
Seizures	-	-	-	-	-	-	0.130	0.140	0.000
Akin. mut.	-	-	-	-	-	-	0.750	0.500	0.440
Resp. dep.	-	-	-	-	-	-	0.150	0.000	0.000

	1 FRONTAL	2 CLIN	3 TEMPORAL	4 CLIN
1 009	0	1	1	1
2 007	1	1	1	0
3 020	0	0	0	1
4 024	0	0	0	1
5 091	1	0	1	0
6 043	0	0	1	1
7 047	0	0	0	1
8 057	0	0	1	1
9 067	0	1	1	1
10 070	0	1	1	1
11 251	1	1	1	1
12 086	1	0	1	1
13 089	1	1	1	0
14 090	1	1	1	1
15 097	0	1	1	1
16 105	1	1	1	1
17 135	1	1	1	1
18 109	0	0	1	1
19 117	1	1	1	1
20 120	0	1	1	0
21 113	1	1	1	0
22 114	1	1	1	0
23 115	1	0	1	1
24 222	0	1	1	0
25 229	0	0	0	1
26 227	1	1	1	1
27 240	0	1	1	1
28 242	1	1	1	0
29 243	0	1	0	1
30 244	1	1	1	1
	1	1	1	1

Legend for Appendix 5:

Comparison of the presence of spongiform change in different areas of the brain with features of neurological dysfunction attributable to that region

A total of 73 cases have been studied: the case reference numbers are given in the left-hand column. The areas considered are frontal lobe, temporal lobe, parietal lobe, basal ganglia, occipital lobe, cerebellum and brainstem. The presence of anti-PrP positive amyloid plaques and ATD pathology have also been recorded.

		5	6	7	8
		PARIETAL	CLIN	BG	CLIN
1	009	0	1	1	1
2	007	1	0	0	1
3	020	1	1	1	0
4	024	0	1	1	0
5	091	1	0	1	0
6	043	1	1	1	0
7	047	0	0	1	1
8	057	1	1	0	1
9	067	0	1	1	0
10	070	1	1	1	1
11	251	0	1	1	0
12	086	0	1	1	1
13	089	1	1	1	1
14	090	1	1	1	0
15	097	0	1	1	1
16	105	0	1	1	0
17	135	1	1	1	1
18	109	0	1	1	1
19	117	0	0	1	0
20	120	0	0	1	0
21	113	1	1	1	0
22	114	1	1	1	0
23	115	1	1	1	0
24	222	0	1	1	0
25	229	1	1	1	0
26	227	1	0	1	1
27	240	1	1	1	1
28	242	1	1	1	1
29	243	0	1	0	1
30	244	1	1	1	0
31	245	0	1	1	0
32	248	1	1	1	0
33	286	0	1	1	0
34	250	0	0	1	1
35	265	0	0	1	0
36	256	1	1	1	1
37	257	1	1	0	1
38	268	1	0	1	1
39	284	1	1	1	0
40	304	1	0	0	0
41	310	1	0	1	1
42	276	1	1	1	1
43	278	1	0	1	0
44	277	1	0	1	1
45	288	1	1	1	1
46	283	0	1	1	0
47	116	1	1	1	0
48	118	1	1	1	1
49	125	1	1	1	0
50	132	0	1	1	0
51	136	1	1	1	0
52	143	1	1	1	0
53	146	0	0	1	0
54	147	1	1	1	1
55	148	1	1	1	1
56	149	1	1	1	0

		9	10	11	12
		OCCIPITA	CLIN	CEREBELL	CLIN
1	009	0	1	1	0
2	007	1	0	0	0
3	020	1	0	1	1
4	024	1	0	1	1
5	091	1	0	1	1
6	043	1	0	1	1
7	047	1	1	1	1
8	057	0	0	1	1
9	067	1	0	1	1
10	070	1	1	1	1
11	251	0	1	1	1
12	086	0	1	1	1
13	089	1	1	1	1
14	090	1	0	1	1
15	097	1	1	1	1
16	105	1	0	1	1
17	135	1	0	1	0
18	109	1	0	1	1
19	117	0	0	1	1
20	120	1	0	1	1
21	113	1	1	1	1
22	114	1	1	1	1
23	115	1	1	1	1
24	222	1	1	1	0
25	229	1	0	1	1
26	227	1	1	1	1
27	240	0	1	1	1
28	242	0	1	0	0
29	243	0	1	1	1
30	244	1	0	1	1
31	245	1	1	1	0
32	248	1	1	1	0
33	286	0	1	1	1
34	250	1	0	1	1
35	265	1	1	1	1
36	256	1	0	1	1
37	257	1	0	0	1
38	268	1	1	1	0
39	284	1	1	1	1
40	304	1	0	1	1
41	310	1	1	1	0
42	276	1	0	1	1
43	278	1	1	1	1
44	277	1	1	1	1
45	288	1	1	1	1
46	283	1	1	1	1
47	116	1	0	1	1
48	118	1	0	1	1
49	125	1	1	1	1
50	132	1	1	1	1
51	136	1	0	1	1
52	143	1	1	1	0
53	146	0	1	1	1
54	147	1	0	1	1
55	148	1	0	1	1
56	149	1	1	1	1

		13 B/STEM	14 CLIN	15 PLAQUES	16 ATD
1	009	0	0	0	0
2	007	0	0	0	0
3	020	1	0	0	0
4	024	0	0	0	0
5	091	0	1	0	0
6	043	0	0	0	0
7	047	0	0	0	0
8	057	0	0	0	0
9	067	0	0	0	0
10	070	0	0	0	0
11	251	0	0	0	0
12	086	0	0	0	0
13	089	0	0	0	0
14	090	0	0	0	0
15	097	0	1	0	0
16	105	0	0	1	0
17	135	0	0	1	0
18	109	1	0	0	0
19	117	0	0	0	0
20	120	1	0	0	0
21	113	0	0	0	0
22	114	1	0	0	0
23	115	1	0	0	0
24	222	0	0	0	0
25	229	0	0	1	0
26	227	0	0	1	0
27	240	0	0	0	0
28	242	0	0	0	0
29	243	0	0	0	1
30	244	0	0	1	0
31	245	0	0	0	0
32	248	0	0	0	0
33	286	0	0	0	0
34	250	0	0	0	0
35	265	1	0	1	0
36	256	0	1	0	0
37	257	1	0	1	0
38	268	0	0	0	0
39	284	0	0	0	0
40	304	0	0	1	0
41	310	0	0	0	0
42	276	0	0	0	0
43	278	0	0	0	0
44	277	0	0	0	0
45	288	0	0	0	0
46	283	0	0	1	0
47	116	0	1	0	0
48	118	0	0	0	0
49	125	1	1	0	0
50	132	0	0	0	0
51	136	1	0	0	0
52	143	0	1	0	0
53	146	0	0	0	0
54	147	0	0	1	0
55	148	0	0	0	0
56	149	0	0	0	0

		1	2	3	4
		FRONTAL	CLIN	TEMPORAL	CLIN
57	161	1	1	1	0
58	155	0	1	0	1
59	157	1	1	1	0
60	164	1	1	1	0
61	254	0	0	0	1
62	194	1	1	1	1
63	174	0	0	0	1
64	193	0	1	1	1
65	196	0	1	1	1
66	197	1	1	1	1
67	205	0	1	0	0
68	207	1	1	1	1
69	220	1	1	1	0
70	208	1	1	1	0
71	223	1	1	1	1
72	305	1	1	1	1
73	285	1	1	1	0

		5	6	7	8
		PARIETAL	CLIN	BG	CLIN
57	161	1	1	1	1
58	155	0	1	1	0
59	157	0	0	1	1
60	164	1	0	1	0
61	254	0	0	1	1
62	194	0	1	1	0
63	174	1	0	1	1
64	193	1	1	1	0
65	196	0	0	1	0
66	197	1	1	1	1
67	205	1	0	1	0
68	207	1	1	1	1
69	220	1	0	1	0
70	208	1	0	1	1
71	223	1	0	1	1
72	305	1	0	1	1
73	285	1	0	1	0

		9	10	11	12
		OCCIPITA	CLIN	CEREBELL	CLIN
57	161	0	0	0	1
58	155	1	0	1	1
59	157	1	1	1	1
60	164	1	1	1	1
61	254	1	1	1	1
62	194	0	0	1	1
63	174	1	1	1	1
64	193	1	0	1	1
65	196	1	1	1	1
66	197	1	1	1	1
67	205	0	0	0	0
68	207	1	0	0	1
69	220	1	1	1	1
70	208	1	0	1	1
71	223	1	0	1	1
72	305	0	0	1	0
73	285	1	1	1	1

		13	14	15	16
		B/STEM	CLIN	PLAQUES	ATD
57	161	0	0	0	0
58	155	0	0	0	0
59	157	0	0	0	0
60	164	0	0	0	0
61	254	0	1	0	0
62	194	0	0	0	0
63	174	0	0	0	0
64	193	0	0	0	0
65	196	0	0	0	0
66	197	0	0	0	0
67	205	0	0	0	0
68	207	0	0	0	0
69	220	0	0	0	0
70	208	0	0	0	0
71	223	0	0	0	0
72	305	0	1	1	0
73	285	0	0	0	0

Legends for Appendices 6 and 7:

Following genetic counselling an information sheet summarising the main points was given to the relatives, and their consent was obtained for PRNP analysis. Relatives were requested to indicate whether the results of genome analysis should be made known to them.

INFORMATION TO BE GIVEN TO RELATIVES OF CJD PATIENTS WHEN CONSENT IS BEING OBTAINED FOR BLOOD TO BE TAKEN FOR GENETIC STUDIES

1. The cause of CJD in the great majority of patients is unknown.

2. A small proportion of cases are hereditary in nature due to a faulty gene.

3. In nearly all the hereditary cases, the family are already aware of other affected family members. In these families about half the family members can be affected by CJD and the disease may occur from generation to generation.

4. The chances of finding a faulty gene in a case of CJD without any other affected family members is very small, probably less than 1 in 50.

5. We wish to take blood from cases of CJD in order to look for abnormalities in the gene and we also store blood for future research.

6. In this way we hope to advance knowledge in CJD which may, in the future, lead to a better understanding of the disease.

7. If you do not want to know the result of this test, we will not inform you, your family doctor or the hospital doctor of the result.

8. If you do want to know the result of the test, this will be done through the local genetic counselling clinic even if the test is negative which is the most likely outcome.

9. The chances of finding an abnormality in the genetic test are very low and in the great majority of cases there is no increased risk of developing this disease in family members. It is particularly important to know that CJD is not infectious and there is no risk from contact with patients during their illness.

10. Summary
    * There is no risk of developing CJD by contact.
    * Only a small proportion of cases are hereditary.
    * The blood sample will help research
    * The result of the genetic test will only be made available IF YOU WANT IT TO BE.

National Creutzfeldt-Jakob Disease Surveillance Unit

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CONSENT FORM FOR GENETIC ANALYSIS IN CJD

I ..... consent to a blood sample being taken from myself/my relative ..... for the purposes of research on the disorder Creutzfeldt-Jakob disease.

Signed .....

The research on the blood sample will principally involve studies that will not have any direct implications for individuals, although we hope that they will help us understand the disorder better in future. However, in a few cases, Creutzfeldt-Jakob disease may result from a change in a genetic factor that could give a risk to family members. In view of this,

1. Would you like to know any test result that might suggest a risk to other family members?

   YES / NO

2. It is possible that information from this research that does not appear to be important now, might become so in the future. If this were to occur, would you like to be informed?

   YES / NO

Signed .....

Signed ..... Research Registrar